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銀耳多醣不織布敷料於大鼠創口癒合之功效[1]

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摘　要

陳鯤兆、吳昭慧、陳忠信、廖俊旺、楊正義、楊淑惠。2012。銀耳多醣不織布敷料於大鼠創口癒合之功效。台灣農業研究 61:1–11。

天然多醣高分子材料，具有良好的生物可分解性與生物相容性，已有商業型敷料於臨床應用。萃取自銀耳 (*Tremella fuciformis*) 子實體的天然高分子聚合物–銀耳多醣，具有保濕及抗發炎之特性。本研究以銀耳多醣不織布為實驗材料，敷貼動物全層皮膚傷口，評估其對但傷口癒合之促進功效，擬作為未來可能臨床應用之參考。結果顯示，試驗期間大鼠的體重在各處理組間均無顯著性差異 ($p > 0.05$)，分析皮膚創口面積結果顯示，陽性對照組敷料 (Kal-PC) 於手術後第10及14天；銀耳多醣敷料組 (Tp-nonW) 則在術後第14天，創口面積相對於陰性對照組 (Alg-NC) 有顯著性減少 ($p < 0.05$)，Tp-nonW組於第10天及第14天之平均癒合面積相對於Alg-NC組分別約增加20%及49.2%，對傷口面積具顯著性縮小，且手術後第14天皮膚創口之上皮再生組織病理總積分，Kal-PC組為5.8分、Tp-nonW組為5.4分相對於Alg-NC組4.6分均呈上升，但因個體差異大，未具統計學顯著性差異 ($p > 0.05$)。本研究顯示，銀耳多醣不織布敷料 (Tp-nonW) 對大鼠傷口具有癒合功效，可做為進一步人體臨床試驗的參考。

關鍵詞：銀耳、多醣不織布、創傷敷料、動物模式。

前　言

皮膚的主要功能包括排泄、保護、吸收、調節、感知等等，因此若皮膚上有傷口時，創傷、褥瘡所造成之傷口，會使皮膚無法發揮正常功能，且影響外觀，甚至引起感染。由於創傷敷材市場極大，因此各國皆有許多相關研究，以原料來看，主要有幾丁質 (chitin)、幾丁聚醣 (chitosan) 、海藻酸 (alginic acid)、透明質酸 (hyaluronic acid)、聚葡萄醣胺基多醣 (glycosaminoglycans) 及硫酸軟骨素 (chondroitin sulfate)，以及上述各種材料的衍生物，此外亦有人工合成材料或上述各種原料的混合物

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(Doillon & Silver 1986; Gao *et al.* 1992; Gilchrist & Martin 1983; Kirker *et al.* 2002; Liu *et al.* 2010; Murakami *et al.* 2010; Wang *et al.* 2006; Wittayaareekul & Prahsarm 2006; Zou *et al.* 2009)。天然多醣高分子材料，具有良好的生物可分解性與生物相容性，已有商業型披覆材於臨床應用。生物相容性敷料主要使用膠原蛋白為原料，而濕潤性傷口癒合產品主要可分為海藻膠、薄膜、泡棉膠、凝膠及水膠，其中薄膜與泡棉膠主要是使用合成的高分子材料，而天然高分子材料則被添加於凝膠及水膠中，或以多層的方式和海藻膠、薄膜等產品結合，成為複合型的產品。皮膚創傷敷料的功能除了遮蓋傷口、減低感染外，更希望能達到加速傷口癒合、讓患者感到舒適及癒合後傷口的疤痕越少越好的目的。銀耳多醣為萃取自銀耳 (*Tremella fuciformis*) 子實體的天然高分子聚合物，具有抗急性輻射中毒 (Zhao *et al.* 1982)、抗發炎 (Ukai *et al.* 1983) 及保濕 (Yang *et al.* 2007) 等特性，以銀耳多醣為材料，可與海藻酸鈉材料製成包括不織布、水膠 (hydrogel) 或多孔體 (sponge) 等敷料產品。

材料與方法

試驗樣品

市售敷料Kaltostat®, Kal-PC，購自ConvaTec公司，作為陽性對照組敷料 (Calcium/Sodium Alginate = 8：2, http://www.dressings.org/Dressings/kaltosta.html)，海藻酸鈉不織布 (sodium alginate nonwoven, Alg-NC) 為陰性對照組及銀耳多醣不織布 (tremella polysaccharides nonwoven, Tp-nonW, 銀耳多醣/海藻酸鈉 = 1：2) 銀耳多醣原料由農試所提供，由財團法人紡織產業綜合研究所試製提供。

試驗動物

雄性大鼠 (SD品系)，購自樂斯科公司，試驗時為8週齡，體重約300 g，分為3組，每組5隻。試驗期間給予充足的飼料及逆滲透飲水，動物飼育室控制狀況為溫度22–25℃，相對溼度50–70% 及12小時光/12小時暗之光照週期。實驗動物之使用與操作均依據中華實驗動物學會之「實驗動物管理與使用指南」規範進行 (Yu *et al.* 2005)。

全層皮膚創口操作

試驗前禁食18小時，以2% Isoflurane (Halocarbon Laboratories, South Carolina, USA) 於實驗動物用麻醉機器 (Benchtop Small Animal Anesthesia Unit, MSS 003, England) 進行吸入麻醉。如圖1A所示，以電剪背部剃毛面積 (長度12 cm × 寬度6 cm)，以背部朝上放置平板上，四肢以膠帶固定，以定形框畫出2 cm × 2 cm點出四角位置，皮膚以優碘及70%酒精先後消毒。框畫出2 cm × 2 cm 面積以手術刀片延線切開背部全層皮膚 (full thickness)，深度約4–5 mm，經鑷子剝皮下脂肪及結締組織，取下皮膚 (Roh *et al.* 2006)。若有出血，以滅菌紗布壓迫止血，取試驗樣品貼於背部傷口，再以無菌紗布、透氣膠布及黏著彈性繃帶包紮固定於背部。止痛措施於大鼠術後給予Rimadyl (Carprofen, Pfizer, NY, USA) 皮下注射5 mg/kg，每天1次，投予3天。

皮膚創傷敷料操作及更換次數

創口以0.9%無菌生理食鹽水清潔，取供試驗敷料，海藻酸鈉不織布敷料、市售Kaltostat®敷料，或銀耳多醣不織布敷料，適量敷料置於傷口，再以透氣膠布 (MicroporeTM, 3M, St. Paul, MN, USA) 及黏著彈性繃帶 (ELATEX, Alcare, Tokyo, Japan) 包紮固定於背部，投藥後在第3、7、10及14天更換敷料 (表1)，並進行觀察及記錄創口變化，並依判定標準加以分析。

評估項目

體重變化：大鼠於手術後3、7、10及14天以天平稱體重及記錄。

創口癒合面積觀察：創口面積計算，如圖1所示，以陰性對照組第3天為例 (圖1A)，用

電荷耦合元件 (charge coupled device, CCD) 攝取各階段皮膚創口影像 (圖1B)，經影像分析儀 (Lieca, Q500MC, Nussloch, Germany) 進行定量分析，創口面積比率 (%) = [創口面積 (圖1C) ÷ 切面總面積 (2 cm × 2 cm) (圖1D)] × 100，求出皮膚創口面積平均指數。

組織病理觀察：術後第14天結束試驗，以2% Isoflurane麻醉大鼠，經腹主動脈採血犧牲動物，取創口中心區皮膚組織之橫切面面積0.5 cm × 2.5 cm (Roh *et al.* 2006) 放入10%的中性福馬林溶液固定1週，經粗修及石臘包埋後製成2 μm厚度之組織切片，分別以H & E及Masson’s trichrome (MT) 方法染色 (Liu *et al.* 1996)，於一般光學顯微鏡下 (Opticphot-2, Nikon, Tokyo, Japan) 觀察皮膚癒合之各種組織病理變化。

皮膚癒合之形態學評估：依據Altavilla *et al.* (2001, 2005) 方法評估項目，表皮上皮細胞

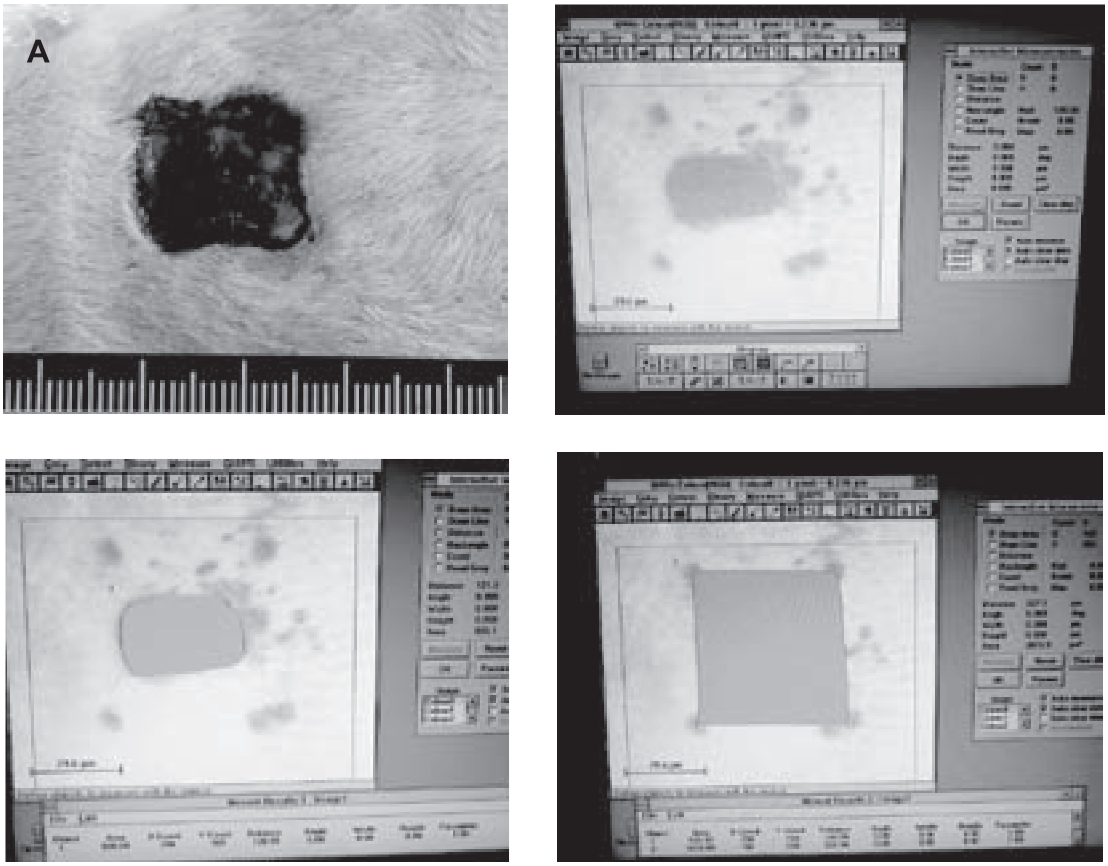

圖 1. 大鼠皮膚創傷敷貼後創口癒合面積之評估。(A) 以陰性對照組第 3 天為例；(B–D) 以電荷耦合元件攝取各階段皮膚創口影像，經影像分析儀進行定量分析。計算其創口面積比率 (%) = [創口面積 (圖 C) ÷ 切面總面積 (2 cm × 2 cm) (圖 D)] × 100，求出皮膚創口面積平均指數。

Fig. 1. Measurement of residual wound area of rat skin after covered Alg-NC (negative control) for 3 days. (A) A photograph showing the wounded area; (B–D) Photographs of wound residual area taken by CCD camera. Note the wound residual area (%) was calculated by the area of wound (C) and divided by total excised area (2 cm × 2 cm) (D) and then multiplied with 100.

表 1. 皮膚創傷敷料更換敷貼次數
Table 1. Time and procedure of treatment of wound dressing on rats

Treatment	Type of wound dressing	Time of changing wound dressing	Procedure of application
Alg-NC (Negative control)	Sodium alginate nonwoven	One time at 3, 7, 10 and 14 days	1. Wound cleaning with 0.9% sterile saline 2. Covering with Sodium alginate nonwoven dressing (2 cm × 2 cm) 3. Covering with gauze (2 inch^2)
Kal-PC (Positive control)	Kaltostat® dressing	One time at 3, 7, 10 and 14 days	1. Wound cleaning with 0.9% sterile saline for 2. Covering with Kaltostat® dressing (2 cm × 2 cm) 3. Covering with gauze (2 inch^2)
Tp-nonW (*Tremella*-polysaccharides)	Tremella polysaccharides nonwoven dressing	One time at 3, 7, 10 and 14 days	1. Wound cleaning with 0.9% sterile saline 2. Covering with Tremella polysaccharides non-woven dressing (2 cm × 2 cm) 3. Covering with gauze (2 inch^2)

再生癒合 (re-epithelialization)、肉芽腫 (granulation)、血管新生 (angiogenesis) 等級予以半定量分析。評估分數為0–4個等級，其中0代表無復原情形 (–, none)、1代表輕微 (+, slight)、2代表中等度 (++, moderate)、3代表完全癒合 (+++, complete) 及4代表顯著 (++++, thick granulation) (表2)。

結　果

體重變化

大鼠在試驗期間之體重於第3天陰性對照組 (Alg-NC)、陽性對照組 (Kal-PC) 及銀耳多醣敷料組 (Tp-nonW) 分別為312.76 g ± 15.25 g、317.56 g ± 11.21 g及311.52 g ± 11.89 g；第7天各組體重分別為323.04 g ± 14.96 g、328.84 g ± 11.89 g及327.60 g ± 20.88 g；第10天各組體重分別為327.44 g ± 17.24 g、330.96 g ± 16.14 g及328.65 g ± 15.61 g；第14天各組體重分別為341.28 g ± 19.09 g、347.36 g ± 9.83 g及336.43 g ± 18.83 g，各組間無顯著性差異 (p > 0.05)。

創口癒合肉眼觀察

觀察試驗敷料皮膚敷料對大鼠皮膚創口於第3、7、10及14天癒合情形。如圖2所示，第3天 (圖2A–C)，各組大鼠皮膚創口及周圍組織仍呈現明顯紅腫，紗布或敷料上仍殘留許多炎症滲出液。以Alg-NC組及Tp-nonW組滲出液相對較多，而Kal-PC組之創口則較為乾燥且表面較為平整。各組皆未見顯著的傷口收縮或上皮再生現象；Kal-PC組及Tp-nonW組可見部分敷料與創口緊密黏合。第7天 (圖2D–F)，各組大鼠皮膚創口及其周圍組織仍呈現明顯紅腫，紗布上仍殘留許多炎症滲出液；各組創口皆開始收縮，創口面積逐漸縮小；可見肉芽腫組織 (granulation tissue) 生成，同時肉芽腫組織呈現潮紅色，顯示亦有血管新生現象。各組大鼠皮膚創口癒合情形以Kal-PC組最佳。第10天 (圖2G–I)，各組大鼠皮膚創口及周圍組織呈紅腫或結痂，紗布上殘留少量炎症滲出液。各組創口面積持續收縮，以Kal-PC組 (圖2H) 及Tp-nonW組 (圖2I) 最為顯著，創口周圍已可見結痂。第14天 (圖2J–L)，各組大鼠皮膚創口依不等程度收縮。以Kal-PC組 (圖2K) 最為顯著，創口區變為狹長，周圍已可見痂皮及毛髮覆蓋。Tp-nonW組 (圖2L) 創口收縮面積介於Alg-NC組及Kal-PC組之間，創口區亦變為狹長，周圍同樣可見痂皮及毛髮覆蓋。

創口面積

以影像系統評估Alg-NC組、Kal-PC組及Tp-nonW組之大鼠皮膚創口於第3、7、10及14天癒合情形。如表3所示結果顯示，第3天

表 2. 大鼠皮膚創口癒合組織病理評估標準

Table 2. Criteria for estimation of wound healing on rats (Based on reports of Altavilla *et al.* 2001, 2005)

Score	Re-epithelialization	Granulation of tissue	Angiogenesis
0, –	Absent	Inflammatory tissue	Absence of angiogenesis, inflammation, and hemorrhage
1, +	Little epidermal and dermal organization	Thin granular layer	Altered angiogenesis (1–2 vessels per site) characterized by a high degree of edema, hemorrhage, and occasional congestion and thrombosis
2, ++	Moderate epidermal and dermal organization	Moderate granulation layer	Few newly formed capillary vessels (3–6 per site), moderate degree of edema and hemorrhage. Occasional congestion and inter vascular fibrin deposition; absence of thrombosis
3, +++	Complete remodeling of epidermis and dermis	Thick granulation layer	Newly formed capillary vessels (7–10 per site), moderate degree of perivascular and interstitial edema and congestion. Absence of thrombosis and hemorrhage
4, ++++		Very thick granulation layer	Newly formed and well- structured capillary vessels (10 per site) vertically disposed toward the epithelium and at the wound margins. Slight degree of perivascular edema

之各組大鼠皮膚創口面積 (residue area) 分別為65.9% ± 8.7%、68.2% ± 10.3%及72.7% ± 3.3%；第7天之各組大鼠皮膚創口面積分別為41.0% ± 13.0%、30.7% ± 5.8%及44.8% ± 6.3%，各組並無顯著性差異；第10天之各組大鼠皮膚創口面積分別為27.5% ± 7.1%、18.9% ± 2.8%及22.0% ± 6.3%，Kal-PC組較Alg-NC對照組創口開始有顯著性縮小 ($p < 0.05$)；第14天之各組大鼠皮膚創口面積分別為19.7% ± 5.7%、6.7% ± 2.8%及10.0% ± 2.7%，Kal-PC組及Tp-nonW組相對於Alg-NC組皆呈現顯著性縮小 ($p < 0.05$)。

癒合面積

創口後之癒合情形如表3所示，第3天之各組大鼠皮膚癒合面積 (healing area) 分別為34.1% ± 8.7%、31.8% ± 10.3%及27.3% ± 3.3%；第7天之各組大鼠皮膚癒合面積分別為59.0% ± 13.0%、69.3% ± 5.8%及55.2% ± 6.3%；第10天之各組大鼠皮膚癒合面積分別為72.5% ± 7.1%、81.1% ± 2.8%及78.0% ± 6.3%，Kal-PC組與Alg-NC組相比具顯著性增加 ($p < 0.05$)；第14天之各組大鼠皮膚已癒合面積分別為80.3% ± 5.7%、93.3% ± 2.8%及90.0% ± 2.7%，Kal-PC組與Tp-nonW組相對於Alg-NC組已癒合面積均有顯著性增加 ($p < 0.05$)。

組織病理評估

判讀大鼠皮膚創傷敷料處理後14天創口癒合之肉芽腫形成組織病理切片圖 (圖3)，肉芽腫形成組織病理積分，Ag-NC組、Kal-PC組、Tp-nonW組分別為1.6分 ± 0.5分、1.8分 ± 0.4分及1.8分 ± 0.4分；創口癒合之上皮再生組織病理積分，各組分別為1.8分 ± 0.4分、2.6分 ± 0.9分及2.2分 ± 0.4分；創口血管新生表現組織病理積分，各組分別為1.2分 ± 0.4分、1.4分 ± 0.5分及1.4分 ± 0.5分；在組織病理總積分，各組分別為4.6分 ± 0.5分、5.8分 ± 0.5分及5.4分 ± 1.0分，如表4所示，各組間無顯著性差異 ($p > 0.05$)。Kal-PC組與Tp-nonW組多數大鼠 (3/5) 創口癒合已完全，而肉芽腫形成組織及血管新生病理積分與Alg-NC組相比則無顯著性差異，推測因為創口癒合進程已經過炎症期和肉芽腫期，進入組織重建期 (structure remodeling) 所致，受損組

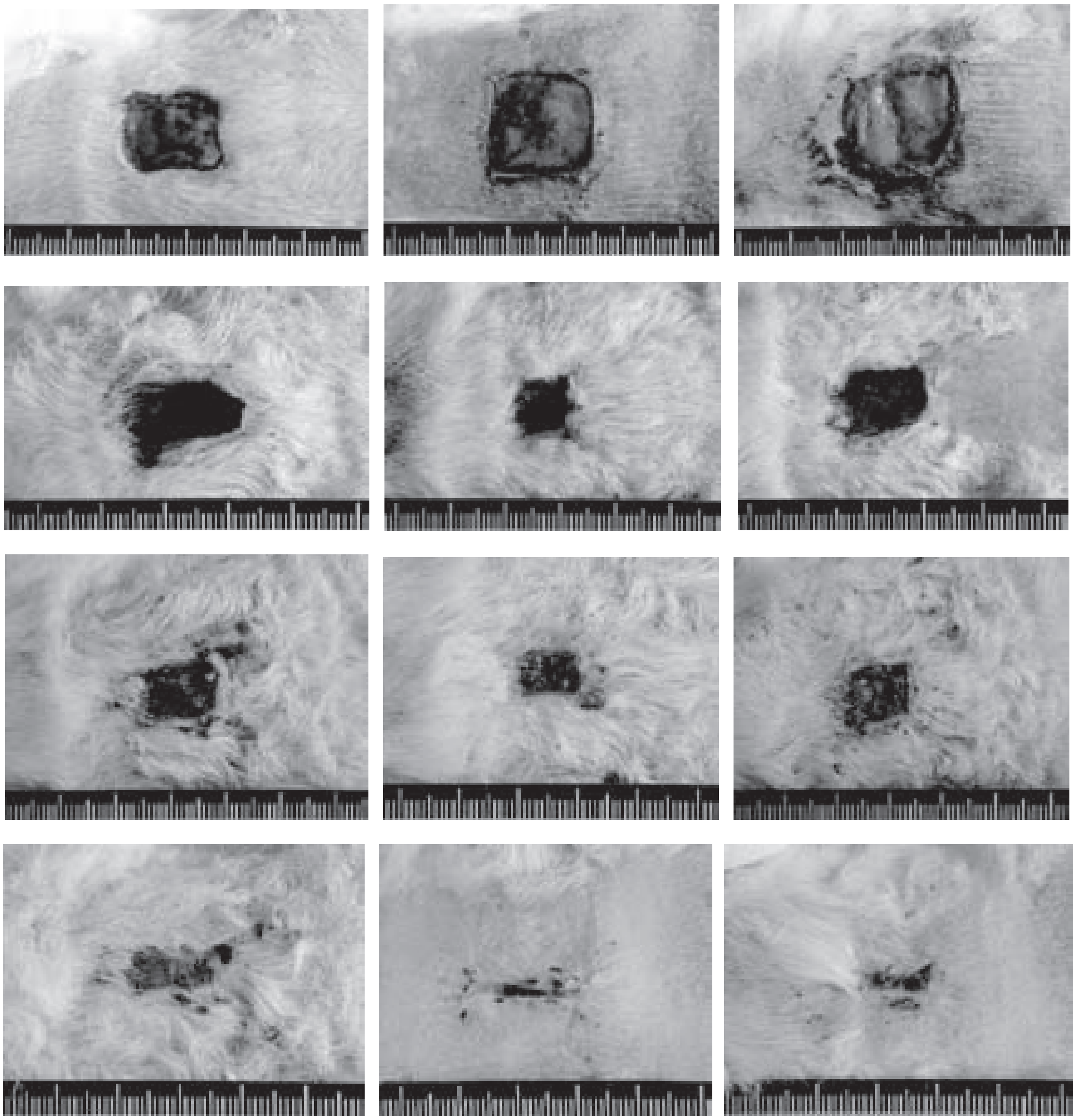

圖 2. 大鼠皮膚創傷敷料敷貼後 3、7、10 及 14 天創口癒合肉眼觀察。第 3 天：(A) 陰性對照組 (Alg-NC)；(B) 陽性對照組 (Kal-PC)；(C) 銀耳多醣敷料 (Kal-PC)。第 7 天：(D) 陰性對照組；(E) 陽性對照組；(F) 銀耳多醣敷料。第 10 天：(G) 陰性對照組；(H) 陽性對照組；(I) 銀耳多醣敷料。第 14 天：(J) 陰性對照組；(K) 陽性對照組；(L) 銀耳多醣敷料。

Fig. 2. Naked eye observation of healing of surgical wounds of rat skin covered with wound dressing for 3, 7, 10 and 14 days. Left column (A, D, G, J): Rats were treated with Alg-NC (Negative control) for 3 (A), 7 (D), 10 (G) and 14 (J) days. Middle column (B, E, H, K): Rats were treated with Kal-PC (Positive control) for 3 (B), 7 (E), 10 (H) and 14 (K) days. Right column (C, F, I, L): Rats were treated with Tp-nonW (Tremella polysaccharide dressing) for 3 (C), 7 (F), 10 (I) and 14 (L) days.

表 3. 各敷料敷貼之大鼠皮膚創口癒合面積
Table 3. Area of residue and healing of each wound dressing covered on surgical wounds of rat skin

Duration of treatment (Days)	Area (%)	Alg-NC [z]	Kal-PC	Tp-nonW
3	Residue [y]	65.9 ± 8.7 [w]	68.2 ± 10.3	72.7 ± 3.3
	Healing [x]	34.1 ± 8.7	31.8 ± 10.3	27.3 ± 3.3
7	Residue	41.0 ± 13.0	30.7 ± 5.8	44.8 ± 6.3
	Healing	59.0 ± 13.0	69.3 ± 5.8	55.2 ± 6.3
10	Residue	27.5 ± 7.1	18.9 ± 2.8*	22.0 ± 6.3
	Healing	72.5 ± 7.1	81.1 ± 2.8*	78.0 ± 6.3
14	Residue	19.7 ± 5.7	6.7 ± 2.8*	10.0 ± 2.7*
	Healing	80.3 ± 5.7	93.3 ± 2.8*	90.0 ± 2.7*

[z] Alg-NC: Negative control; Kal-PC: Positive control; Tp-nonW: Tremella polysaccharide dressing.
[y] Wound residual area (%) was calculated by the area of wound and divided with total excised area (2 cm × 2 cm) and then multiplied with 100.
[x] Wound healing area (%) was calculated by the formula: Healing area (%) = (total excised area of wound)/total excised area (2 cm × 2 cm) × 100.
[w] Data was expressed as Mean ± SD ($n = 5$).
* Significant difference between the control and treated groups at $p < 0.05$.

織從肉芽腫組織轉為較為成熟的膠原沈積 (mature collagen deposition)，並已形成瘢疤 (scar)。

討 論

以大鼠 (SD rats) 於背部以定形框畫出2 cm × 2 cm，以手術刀片延線切開並取下背部全層皮膚 (full thickness)。取供試驗敷料貼敷於背部傷口，再以透氣膠布及黏著彈性繃帶包紮固定，手術後在第3、7、10及14天進行傷口癒合觀察及記錄，並依判定標準加以分析。結果顯示，實驗期間各敷料組之大鼠體重及增重比率 (%) 無顯著性差異 ($p > 0.05$)。於第3、7、10及14天進行之皮膚創口癒合面積量化及組織病理分析顯示，Kal-PC組於術後10及14天在創口面積相對於Alg-NC組有顯著性下降 ($p < 0.05$)；Tp-nonW組在術後第10天之平均創口面積為Alg-NC組面積的80.0%，術後第14天之平均創口面積為Alg-NC組面積之50.8%，具有統計學上顯著性差異 ($p < 0.05$)；從已癒合面積比較，Tp-nonW組在術第10天及第14天之平均癒合面積較Alg-NC組分別增加20.0%及49.2%，亦具有統計學上顯著性差異 ($p < 0.05$)。組織病理評估第14天皮膚創口癒合指數，病理總積分Kal-PC組為5.8分，Tp-nonW組總積分為5.4分，相對於Alg-NC組4.6分雖呈現上升趨勢，但因個體差異較大，未具統計學上顯著性差異 ($p > 0.05$)。

此外，在組織病理學觀察下可見Alg-NC組及Tp-nonW組在肉芽腫組織中有大量不染性斷片沉積，而Kal-PC組亦有部分不染性斷片沉積 (圖3)，比對肉眼觀察結果，敷料與創口有緊密黏合情形。曾有報告指出，使用海藻酸鹽為基質敷料治療較無感染狀況的病人，可以發現較為顯著的異物肉芽腫性反應，並有報告指出此反應可能在約12週後完全消退 (Matthew *et al.* 1993; Odell *et al.* 1994)，不過在臨床上認為此藻酸鹽敷料特性雖在創傷初期形成一種不可溶性的纖維斷片，但在止血期的過程中進行離子交換後，可以變成水溶性膠體樣結構，最後可以被身體正常的生理功能所移除 (Berry *et al.* 1996)。在本次試驗中，可見Tp-nonW組及Kal-PC組的斷片周圍具有成熟的纖維細胞與小微血管叢圍繞，證明此反應可以促進創傷區域的血管重新形成，且雖在新生肉芽組織內存在

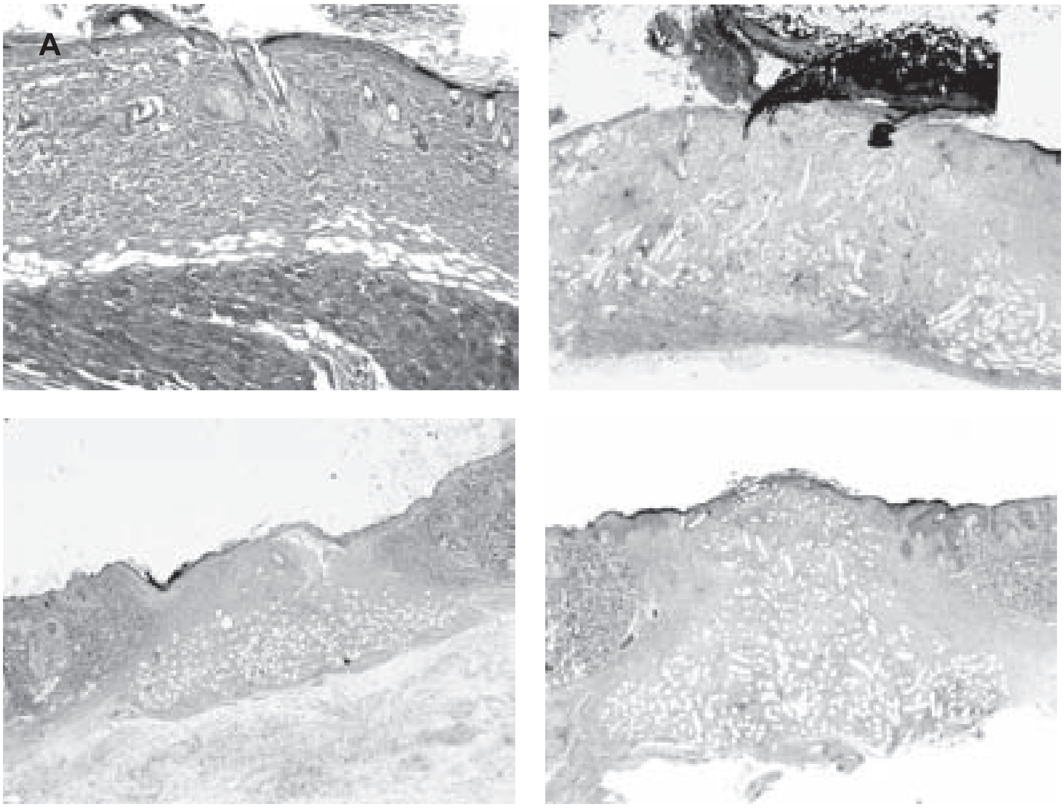

圖 3. 各敷料敷貼 14 天後大鼠創口癒合之肉芽腫形成組織病理切片。(A) 正常皮膚；(B) 陰性對照組皮膚 (Alg-NC)；(C) 陽性對照組皮膚 (Kal-PC)；(D) 銀耳多醣敷料 (Tp-nonW)。Masson's Trichrome staining, 40×。

Fig. 3. Histopathology of healing granuloma formed on surgical wounds of rat skin after 14 days of covered with wound dressing of Noraml skin (A), Alg-NC (B), Kal-PC (C) or Tp-nonW (D). Magnification: 40×.

表 4. 各敷料敷貼之大鼠皮膚創口組織病理評估

Table 4. Pathological scores of healing of wounded tissues of rats by the treatment of wound dressing

	Type of wound dressing		
Pathological score	Alg-NC [z]	Kal-PC	Tp-nonW
Epidermal and dermal regeneration	1.8 ± 0.4 [y]	2.6 ± 0.9	2.2 ± 0.4
Granulation tissue thickness	1.6 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
Angiogenesis	1.2 ± 0.4	1.4 ± 0.5	1.4 ± 0.5
Mean score	4.6 ± 0.5	5.8 ± 0.5	5.4 ± 1.0

[z] Alg-NC: Negative control group; Kal-PC: Positive control; Tp-nonW: Tremella polysaccharide dressing.

[y] Data was expressed as Mean ± SD ($n = 5$).

大量的纖維斷片，但其新生的肉芽組織是較為緻密且成熟的纖維結締組織。同時本次試驗皮膚創口較為乾燥，推測可能延遲敷料基質的吸收時間。銀耳多醣不織布製作原料有銀耳多醣、海藻酸鈉及氯化鈣等，日後製作銀耳多醣不織布敷料時，可更改敷料基質的比例 (Panchatcharam *et al.* 2006; Roh *et al.* 2006) 加強銀耳多醣不織布敷料促進傷口癒合的速率。

結　論

以動物創口癒合試驗之全層創口皮膚試驗期中，從已癒合面積 (%) 方面比較，第10天之各組大鼠皮膚已癒合面積，陽性對照組 (Kal-PC) 與陰性對照組 (Alg-NC) 相比具顯著性上升 ($p < 0.05$)；第14天則陽性對照組、銀耳多醣敷料組與陰性對照組相比皆具顯著性上升 ($p < 0.05$)。對大鼠創口皮膚之上皮再生組織病理積分，三組皮膚敷料中依序為陽性對照組 (市售敷料Kaltostat®, Kal-PC) > 銀耳多醣不織布敷料組 (Tremella polysaccharides nonwoven, Tp-nonW) > 陰性對照組 (Sodium alginate nonwoven, Alg-NC)。

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Effect of *Tremella* Polysaccharides Nonwoven Dressing on Healing of Surgical Wounds of Rats[1]

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Abstract

Chen, K. C., J. H. Wu, C. H. Chen, J. W. Liao, C. Y. Yang, and S. H. Yang. 2012. Effect of *Tremella* polysaccharides nonwoven dressing on healing of surgical wounds of rats. J. Taiwan Agric. Res. 61:1–11.

Some commercial products used as wound dressing in clinics are made of natural polysaccharides which are biodegradable and biocompatible. Polysaccharides extracted from fruiting bodies of *Tremella fuciformis* are nature polymers with moisturizing and anti-inflammatory properties. The objective of this study was to determine effects of the *Tremella*-polysaccharide non-woven product (Tp-nonW) as wound dressing on healing of surgical wounds of rats. Results showed that wound dressing with Tp-nonW or Kal-PC (a commercial product Kaltostat® made of Calcium/Sodium Alginate and used as positive control) significantly ($p < 0.05$) decreased the wound area of rats. Compared to the treatment of wound dressing of Alg-NC (a commercial product made of Sodium Alginate and used as negative control), the healing area of wounds covered with Tp-nonW for 10 and 14 days increased by 20 and 49.2% respectively. Results of the histopathological study showed that the score of regrowth tissues from wounded skin was 5.8 and 5.4 for the treatment of Kal-PC and Tp-nonW, respectively, compared to the score of 4.6 for the negative control Alg-NC, but the difference among these three treatments was statistically insignificant ($p > 0.05$). This study suggests that *Tremella*-polysaccharide non-woven dressing (Tp-nonW) has wound healing properties on rats and it may warrant further studies in human clinic trials.

Key words: *Tremella fuciformis*, Polysaccharides nonwoven, Wound dressing, Animal model.

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台灣原生枇杷之遺傳親緣關係及分類研究 [1]

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摘　要

張坤城、邱輝龍、曾彥學、鄧書麟。2012。台灣原生枇杷之遺傳親緣關係及分類研究。台灣農業研究 61:12–28。

台灣原生枇杷屬 (*Eriobotrya*) 植物是枇杷作物的野生近緣種，廣泛分布於整個台灣。過去有被歸類為2個不同種、或3個不同變種、或3個不同型或被建議歸類為2個變種1個型。本研究利用核醣體DNA內轉錄區 (internal transcribed spacers, ITS) 序列、葉綠體內*matK*基因部分序列及葉部、花序與花粉粒等形態性狀探討台灣原生枇杷屬之分類地位。結果顯示，14份台灣原生枇杷屬植物均可獲得593個鹼基對 (base pair) 的ITS序列，僅有7個變異位點 (variation sites)，而*matK*基因部分序列658個鹼基對中並無任何的差異位點；以鄰接法所建構的親緣關係樹 (phylogenetic tree) 指出台灣原生枇杷屬植物為單源群 (monophyletic group)，然卻無法有效區分台灣枇杷、武威山枇杷及恆春山枇杷等種下分類群。綜合前述資料表明台灣原生枇杷為同一個種，至於種下的分類群則有待其他分子層次的資料來釐清。

關鍵詞：台灣枇杷、薔薇科、ITS序列、*matK*序列、親緣關係。

前　言

枇杷屬 (*Eriobotrya*) 為薔薇科 (Rosaceae) 梨族 (Pyreae) 常綠喬木植物，全屬約有32種，分布於亞洲溫帶及亞熱帶地區 (Badenes *et al.* 2009; Li *et al.* 2011a; Lin *et al.* 2004)。其中枇杷 [*Eriobotrya japonica* (Thunb.) Lindl.] 為重要的經濟果樹 (Badenes *et al.* 2009)，台灣多栽培於中部海拔200–1000 m之坡地 (Shih 2007)。

台灣原生的枇杷最早由 Hemsley (1895) 發表為石楠屬 (*Photinia*)，學名為 *Photinia deflexa* Hemsl. (台灣枇杷)，模式標本採自台灣；之後Henry (1896) 紀錄台灣有兩種，即 *P. deflexa* Hemsl.及栽培種枇杷 [*E. japonica* (Thunb.) Lindl.]。而Matsumura & Hayata (1906) 及Kawakami (1910) 亦均有*P. deflexa* Hemsl. 的紀錄。Hayata (1913) 則發表一新種*P. buisanensis* Hayata (武威山枇杷)。Nakai (1916) 將*P. deflexa* Hemsl.轉移至枇杷屬中，並將*P.*

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buisanensis Hayata新組合為其型 (forma)，即*E. deflexa* (Hemsl.) Nakai f. *buisanensis* (Hayata) Nakai。Kanehira (1917) 仍沿用*P. deflexa* Hemsl.及*P. buisanensis* Hayata。Sasaki (1928) 則使用*E. deflexa* (Hemsl.) Nakai及*E. buisanensis* (Hayata) Kanehira。Kanehira (1936) 將武威山枇杷歸類為台灣枇杷變種 (variety) *E. deflexa* (Hemsl.) Nakai var. *buisanensis* (Hayata) Kanehira & Sasaki，並發表一新變種*E. deflexa* (Hemsl.) Nakai var. *koshunensis* Kanehira & Sasaki (恆春山枇杷)。Masamune (1954) 將武威山枇杷及恆春山枇杷歸類為台灣枇杷的型。Li (1963) 將台灣枇杷分為三個型，即*E. deflexa* (Hemsl.) Nakai f. *deflexa* (台灣枇杷)、*E. deflexa* (Hemsl.) Nakai f. *buisanensis* (Hayata) Nakai (武威山枇杷) 及*E. deflexa* (Hemsl.) Nakai f. *koshunensis* (Kanehira & Sasaki) H. L. Li (恆春山枇杷)，在此之後台灣枇杷大致沿用Li (1963) 的分類。Liu (1972) 及Yu *et al.* (1974) 均使用同Li (1963) 的學名歸類。Liu & Su (1977) 將台灣枇杷處理為兩個型，即台灣枇杷及武威山枇杷，將恆春山枇杷歸類為台灣枇杷的異名 (synonym)。Ying (1985) 訂正台灣產薔薇科植物時，沿用Li (1963) 的歸類將台灣枇杷分為三個型。Ohashi (1993) 將台灣枇杷分為兩個型，將恆春山枇杷歸類為台灣枇杷的異名 (synonym)。Liu *et al.* (1994) 及Lu *et al.* (2000) 則將台灣枇杷分為三個型，即台灣枇杷、武威山枇杷及恆春山枇杷。Lu *et al.* (2003) 另將武威山枇杷及恆春山枇杷歸類為台灣枇杷的異名不分變種或型。Hong *et al.* (2009) 以逢機擴大多型性去氧核醣核酸標誌 (RAPD) 分析台灣原生枇杷族群之遺傳變異，結果將台灣枇杷與恆春山枇杷分為兩型，武威山枇杷因遺傳距離與栽培種枇杷較近而建議處理為變種。

整理過去台灣原生枇杷屬的分類歸類 (表1) 可以發現台灣枇杷及其種下分類群的分類歸類至今仍存在許多的紛歧。由於過去的分類歸類主要以外部形態作為依據，近年來已有利用SSR (Soriano *et al.* 2005)、RAPD (Hong *et al.* 2009; Yang *et al.* 2007a, 2009b)、ISSR (Xie *et al.* 2007)、AFLP (Yang *et al.* 2007a, 2009a) 與Sequence-Related Amplified Polymorphism (SRAP) (Qiao *et al.* 2011) 等分子標誌或DNA片段序列 (Li *et al.* 2007, 2009, 2011b; Yang *et al.* 2011) 進行枇杷屬的分類研究與遺傳歧異度分析。

核糖體RNA基因 (ribosomal RNA gene, rDNA) 片段之內轉錄間隔區內 (internal transcribed spacer, ITS) 序列與長度在物種間差異很大，已廣泛應用於近緣物種間親緣關係之研究 (Alvarez & Wendel 2003; Baldwin 1992; Baldwin *et al*. 1995; Hamby & Zimmer 1992; Li *et al.* 2009; Yang *et al.* 2011)，而葉綠體中的*matK*基因長度約1500 bp，位於*trnK*基因的插入子 (intron) 區域 (Hilu & Liang 1997)，是葉綠體基因組中演化最快速的編碼區域 (Neuhaus & Link 1987; Olmstead & Plamer 1994)，適合做為植物親緣關係分析的依據 (Johnson & Soltis 1995; Kron 1997; Liang & Hilu 1996; Plunkett *et al*. 1997; Steel & Vilgalys 1994)。

隨著經濟發展、人口增加與氣候變遷等，台灣地區許多野生種原的棲息地已遭破壞，益顯野生種原保育的重要性，而分類地位的釐清，則為種原保育所必需 (Mac 2004)。本研究即針對台灣原生枇杷屬植物進行親緣關係及分類上的整合研究，希望藉此研究作為台灣原生枇杷屬分類鑑定及保育的依據。

材料與方法

試驗材料

台灣原生枇杷材料採自野外之新鮮標本 (圖1，表2)，引證標本存放於中興大學森林系標本館 (TCF) 中。除採集之材料外並查閱國內

表 **1.** 台灣枇杷之學名處理沿革

Table 1. History of taxonomic treatment of native *Eriobotrya* in Taiwan

Year	Author	*Eriobotrya deflexa* f. *deflexa*	*Eriobotrya deflexa* f. *buisanensis*	*Eriobotrya deflexa* f. *koshunensis*
1895	Hemsley	*Photinia deflexa* n. s. [z]		
1896	Henry	*P. deflexa*		
1906	Matsumura & Hayata	*P. deflexa*		
1910	Kawakami	*P. deflexa*		
1913	Hayata	*P. deflexa*	*Photinia buisanensis* n. s.	
1916	Nakai	*E. deflexa* f. *deflexa* *n. c.*	*E. deflexa* f. *buisanensis* n. c.	
1917	Kanehira	*P. deflexa*	*P. buisanensis*	
1928	Sasaki	*Eriobotrya deflexa*	*Eriobotrya buisanensis*	
1936	Kanehira	*E. deflexa* var. *deflexa*	*E. deflexa* var. *buisanensis* n. c.	*E. deflexa* var. *koshunensis* n. var.
1954	Masamune	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
1963	Li	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
1972	Liu	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
1974	Yu *et al.*	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
1977	Liu & Su	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	syn. of *E. deflexa*
1985	Ying	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
1993	Ohashi	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	syn. of *E. deflexa*
1994	Liu *et al.*	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
2000	Lu *et al.*	*E. deflexa* f. *deflexa*	*E. deflexa* f. *buisanensis*	*E. deflexa* f. *koshunensis*
2003	Lu *et al.*	*E. deflexa* f. *deflexa*	syn. of *E. deflexa*	syn. of *E. deflexa*
2009	Hong *et al.*	*E. deflexa* var. *deflexa*	*E. deflexa* var. *buisanensis*	*E. deflexa* f. *koshunensis*

[z] c.: combination; n.: new; f.: forma; s.: species; syn.: synonym; var.: variety.

各標本館中所典藏之臘葉標本，收藏於國外之模式標本則透過網路資料庫查閱照片影像檔。

本研究檢視之標本館及其國際代號包括：(1) 中央研究院植物研究所標本館，台灣、台北 (HAST)；(2) 行政院農業委員會林業試驗所植物園標本館，台灣、台北 (TAIF)；(3) 國立台灣大學生命科學系標本館，台灣、台北 (TAI)；(4) 國立中興大學森林系標本館，台灣、台中 (TCF)；(5) 國立自然科學博物館標本館，台灣、台中 (TNM)；(6) 國立屏東科技大學森林資源管理技術系標本館，台灣、屏東 (PPI)。

花粉形態觀察

將採集到的新鮮標本花朵以FAA液浸泡固定，經過30%、50%、70%、80%、90%、95%酒精序列脫水處理 (每處理20分鐘)，然後以解剖針將花藥中之花粉粒挑出，靜置於放有矽膠乾燥劑之乾燥箱內隔夜，再將乾燥後之花粉 沾黏在貼上錫箔膠帶的載檯 (stab) 上之後將花粉以鍍膜儀鍍金 (coating) 90秒 (厚度約20 nm)，再以掃描式電子顯微鏡 (Scanning Electron Microscope, SEM) (HITACHI S-3000N, Japan) 觀察花粉粒的形態，使用加速電壓15 kv進行照片拍攝。

親緣關係分析

植物總DNA的抽取：每樣品取2.5 g葉片，依照Gawel & Jarret (1991) 之流程並做適當調整之CTAB方法抽取植物總DNA，並貯存於-20℃冰箱中備用。DNA濃度以分光光譜儀 (HITACHI, model 3000, Japan) 測定。

ITS序列之擴增：ITS區域片段序列的擴

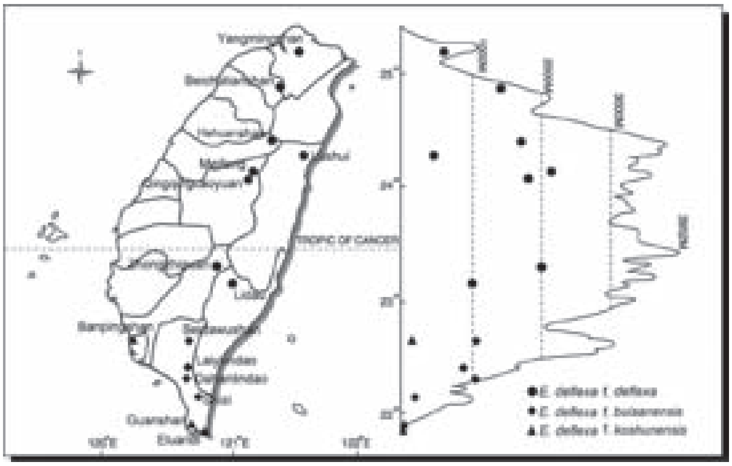

圖 1. 本研究採集材料地理分布圖。

Fig. 1. Samples of native *Eriobotrya* in Taiwan collected for use in this study. Note differences in latitudinal and altitudinal distributions among these samples.

表 2. 參試樣本清單

Table 2. List of native *Eriobotrya* samples used in this study

Taxon	Collection No./GenBank Accession No.	Collection site	Code/Note
Eriobotrya deflexa f. *deflexa*	C4624	Yangmingshan, Taipei	01D
	C4678	Beichatianshan, Taoyuan	02D
	CHL0001	Hehuanshan, Taichung	03D
	C0130	Meifeng, Nantou	04D
	C0134	Qingqingcaoyuan, Nantou	05D
	C4645	Lushui, Hualian	06D
	C4622	Lidau, Taidong	07D
Eriobotrya deflexa f. *buisanensis*	C1140	Beidawushan, Pingtung	01B
	C4649	Laiyilindao, Pingtung	02B
	C2325	Dahanlindao 9K, Pingtung	03B
	C4713	Shizi, Pingtung	04B
Eriobotrya deflexa f. *koshunensis*	C0001	Banpingshan, Kaohsing	01K
	C4615	Guanshan, Pingtung	02K
	C4616	Eluanbi, Pingtung	03K
Eriobotrya japonica cv. Bianco	FJ449734	NCBI [z]	ITS
Eriobotrya bengalensis	FJ571503	NCBI	ITS
Eriobotrya cavaleriei	FJ810022	NCBI	ITS
Eriobotrya fragrans	FJ810024	NCBI	ITS
Eriobotrya seguinii	FJ571507	NCBI	ITS
Photinia serratifolia	FJ810021	NCBI	ITS
Eriobotrya japonica	GQ434196	NCBI	*mat*K
Photinia serratifolia	AM288111	NCBI	*mat*K

[z] NCBI: National Center for Biotechnology Information.

增採用ITSL與ITS4引子對，其序列分別為ITSL：TCGTAACAAGGTTTCCGTAGGTG (Hsiao *et al.* 1995) 及ITS4：TCCTCCGCTTATTGATATGC (White *et al.* 1990)。PCR的反應條件為100 ng模板DNA，0.3 µM引子對，200 µM dNTPs (Stratagene, Germany)，1.5 mM $MgCl_2$，1.0單位的*Taq* DNA聚合酶 (polymerase) 及1× PCR buffer (Phusion, Finnzymes, Finland)，反應總體積為50 µL。擴增 (amplification) 步驟依產品說明為98℃下30秒初變性 (initial denaturing)，接著98℃10秒，鍊合時間為49℃20秒，72℃30秒，連續進行35循環，最後以72℃7分鐘結束反應。所有反應均在熱循環反應器 (thermocycler) 進行 (TC-9600-G, Labnet International Inc. Edison, NJ, USA)，產物以1.5%瓊膠 (Seakem LE Agarose Cambrex Bio Science Rockland Inc., ME, USA) 在0.5× TBE buffer (BDH, England) 中電泳分離。電泳條件為在室溫下以100 V電壓分離35–40分鐘 (Mupip, Tokyo, Japan)。分子量標誌為採50 bp ladder (Promega, USA)。電泳完後以10 mg/mL溴化乙錠 (ethidium bromide, EtBr) (BDH, England) 染色，並在260 nm波長之紫外光中，觀察DNA條帶，並以影像分析系統進行照相及存檔，再以熱感相片紙輸出記錄。

***matK*序列之擴增**：採用Hu *et al.* (2000) 所設計的引子進行擴增反應，其中*matK*-forward的序列為CGTAAACAGTCTTCTTATTTACG，*matK*-backward的序列為TATGTTTACGAGCCAAAGTTCTA。PCR的反應條件則參考Potter *et al.* (2002) 發表的方法。反應條件為50 ng模板DNA，1.0 µM引子對，200 µM dNTPs (Stratagene, Germany)，1.5 mM $MgCl_2$，1.0單位的*Taq* DNA聚合酶及1× PCR buffer (Invitrogen, USA)，反應總體積為25 µL。擴增步驟為95℃下1.5分鐘初變性，接著95℃30秒，鍊合時間為42℃30秒，72℃1.5分鐘，連續進行40循環，最後以72℃10分鐘結束反應。其餘方法與條件與ITS序列增幅反應相同。

擴增片段之選殖與分析：以等量pGEM®-T vector、2× ligation buffer及T4 DNA ligase (Promega, WI, USA)，加水至體積為10 µL，置於16℃下4小時進行接合反應 (ligation)。取5 µL已完成接合反應的溶液，加入50 µL勝任細胞 (competent cell) (*E. coli* DH5α)，緩慢混合均勻後置於0℃下，再以42℃水浴進行熱休克 (heat shock)，接著加入S.O.C Medium於37℃培養1小時，然後塗布於含有IPTG、X-gal及Ampicillin之LB (Luria-Bertani Broth, Miller) 篩選培養基上，置於37℃下培養過夜。

選殖菌株之篩選與確認：挑取篩選培養基上白色菌落，利用聚合酵素連鎖反應 (Econo-Taq® 2X Master Mix, Lucigen, WI. USA) 及電泳挑選出分子量正確之選殖菌株，進一步利用限制酵素*EcoR* I (BioLabs, New England) 將插入DNA片段兩端切下，經電泳再次確認此DNA片段能與質體接合及隨質體複製。然後進行質體的抽取與純化利用 (miniprep purification kit, Protech., USA)，並以電泳判定其品質。每一樣品均選取5個經確認之選殖菌株送交台灣波仕特生物科技股份有限公司進行解序，以ABI PRISM® 377 DNA Sequencer定序，並將5次結果進行相互驗證。

DNA序列資料分析：參試樣品之序列先以BioEdit (v.7.0.9, Tom Hall, Ibis Bioscience, CA, USA) 軟體進行對齊與多重序列排序 (multiple sequence alignment)，再利用Molecular Evolutionary Genetics Analysis (MEGA v.4.0) (Tamura *et al.* 2007) 軟體進行親緣關係分析及平均遺傳距離的計算，並從National Center for Biotechnology Information (NCBI) 下載之栽培種枇杷 (*E. japonica*) 與石楠 (*P. serratifolia*) 之ITS與*matK*序列，及栽培種枇杷品種 (*E. japonica* cv. 'Bianco') 與4份同屬不同種的南亞枇杷 (*E. bengalensis*)、大花枇杷 (*E. cavaleriei*)、香花

枇杷 (*E. fragrans*) 及小葉枇杷 (*E. seguinii*) 的ITS序列分別當做外群進行比對 (表2)。任兩序列間的距離 (pairwise distance) 以Kimura's two-parameter模型 (Kimura 1980) 計算，再以complete deletion方式建構鄰接樹 (Neighbor-joining tree, NJ tree) (Saito & Nei 1987)，並進行interior branch test重複1000次檢定樹型分枝 (node) 的可信度 (Sitnikova *et al.* 1995)。

結　　果

花粉形態觀察

台灣原生枇杷屬植物之花粉均為近球形或至略扁球形，且為三溝孔粒 (tri-colporate)，內孔為長方形，溝較長，兩端較窄；極面觀則呈三裂圓形 (tri-lobed circular)。極軸 (polar axis) 長約為21.2–25.1 μm，赤道軸 (equatorial axis) 長約為22.0–26.3 μm。外壁紋飾有皺波及散條紋，具孔穴至無孔穴 (圖2–4)。

台灣枇杷 (*E. deflexa* f. *deflexa*) 的花粉 (圖2) 極軸長約25.1 μm，赤道軸長約26.3 μm，PE比值為0.95，外壁紋飾為皺波有淺散條紋無刺穿孔 (表3)；武威山枇杷 (*E. deflexa* f. *buisanensis*) 的花粉 (圖3) 極軸長約22.3 μm，赤道軸長約22.8 μm，PE比值0.98，外壁紋飾為較深之散條紋具稀疏刺穿孔 (表3)；恆春山枇杷 (*E. deflexa* f. *koshunensis*) 的花粉 (圖4) 極軸長約21.2 μm，赤道軸長約 22.0 μm，PE比值0.96，外壁紋飾為較深之散條紋具明顯較多的刺穿孔(表3)。

台灣枇杷外壁紋飾為皺波有淺散條紋無刺穿孔，與武威山枇杷、恆春山枇杷的外壁紋飾有較深的散條紋且具刺穿孔可以區別。另武威山枇杷及恆春山枇杷的刺穿孔分別為稀疏的刺穿孔及明顯較多的刺穿孔，亦可作為區分之依據 (表3)。

親緣關係分析

ITS DNA序列親緣關係分析：每一群各選取5個再次確認之選殖菌株進行解序，這些選

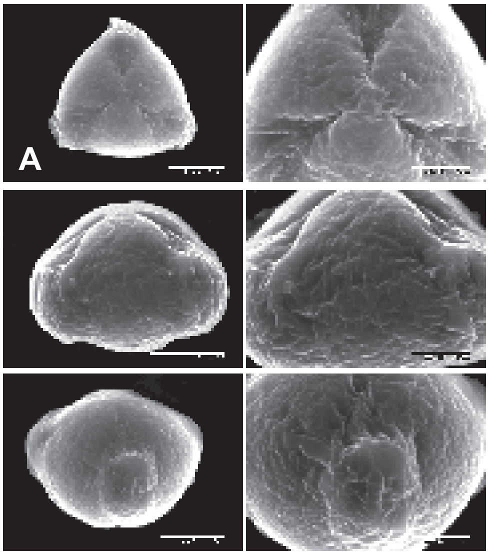

圖 2. 台灣枇杷花粉的掃描式電子顯微鏡照片。(A、B) 極面；(C、D) 赤道面；(E、F) 溝孔。

Fig. 2. SEM-micrographs of pollen grains of *Eriobotrya deflexa* f. *deflexa*, showing polar view (A, B), equatorial view (C, D), and colporate view (E, F).

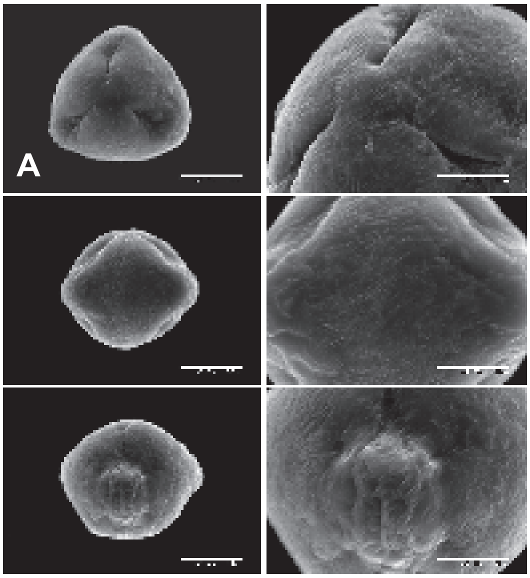

圖 3. 武威山枇杷花粉花粉的掃描式電子顯微鏡照片。(A、B) 極面；(C、D) 赤道面；(E、 F) 溝孔。

Fig. 3. SEM-micrographs of pollen grains of *Eriobotrya deflexa* f. *buisanensis*, showing polar view (A, B), equatorial view (C, D), and colporate view (E, F).

殖菌株經定序後，以BioEdit軟體進行對齊排列，產生一致性序列 (consensus sequences)，並參考NCBI網站中序列比對資料庫 (BLAST) 中栽培種枇杷 (*E. japonica*) Bianco品種的序列 (GenBank accession number: FJ449734) 區分其ITS1、ITS2及5.8S的序列。本試驗綜合14份台灣原生枇杷材料之ITS一致性序列分別獲得593鹼基對 (base pair, bp)，其中ITS1的序列長度均為223 bp，GC含量為64.57–65.02%，僅出現1個變異位點 (variation sites) (位於205 bp)；ITS2

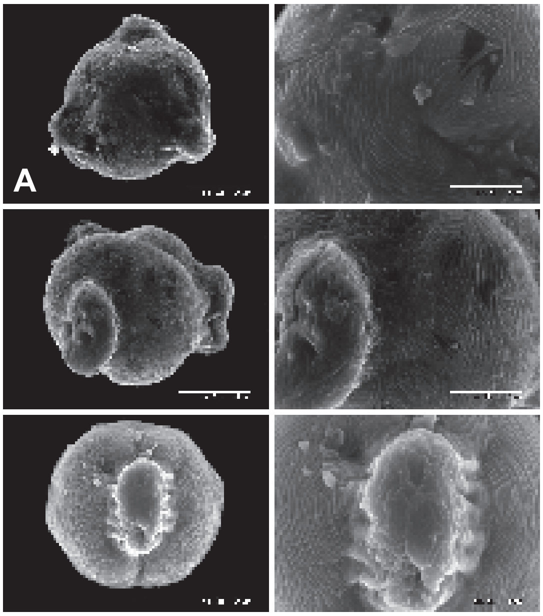

圖 4. 恆春山枇杷花粉的掃描式電子顯微鏡照片。(A、B) 極面；(C、D) 赤道面；(E、F) 溝孔。

Fig. 4. SEM-micrographs of pollen grains of *Eriobotrya deflexa* f. *koshunensis*, showing polar view (A, B), equatorial view (C, D), and colporate view (E, F).

的序列長度為204 bp，GC含量為69.12–70.59%，出現6個變異位點 (分別位於78 bp, 94 bp, 96 bp, 164 bp, 176 bp, 198 bp)；而5.8 S的序列長均為166 bp，GC含量均為65.45%，無變異位點 (表4)。

以本試驗14份台灣原生枇杷及NCBI下載枇杷屬植物之ITS序列建構各序列間之親緣關係樹 (phylogenetic tree)，並依Kimura's twoparameter (Kimura 1980)方法計算出距離指數，由此矩陣為基礎並以1份栽培種枇杷、4份原生於中國大陸同屬不同種的枇杷及1份石楠 (表2) 為外群建構鄰接樹 (NJ tree) (圖5)，並以interior branch test重複1000次評估各分枝點的可信度。結果指出各地採集的台灣原生枇杷聚結為一群，其可信度高達96%，與其他參考材料分開。

參試的三份恆春山枇杷樣品，其中採自南部鵝鑾鼻的恆春山枇杷與採自北部北插天山、中部梅峰和合歡山的台灣枇杷群聚為第一群；而採自北大武山及關山的另外二份樣品則與採自南迴公路獅子鄉路段、來義林道與北大武山的武威山枇杷及採自台東利稻的台灣枇杷樣品無差異而成一群，另一份參試的武威山枇杷是採自大漢林道9公里處，卻與採自北部陽明山的台灣枇杷聚結為一群，此二群再與採自花蓮綠水步道的台灣枇杷聚結為第二群；採自中部青青草原的台灣枇杷單獨自成一群而為第三群。

計算台灣原生枇杷三群間平均遺傳距離 (average pairwise genetic distance) (表5) 指出，

表 3. 台灣原生枇杷之花粉形態

Table 3. Pollen morphology of native *Eriobotrya* in Taiwan

Taxon	Polar axis (P)/μm	Equatorial axis (E)/μm	P/E	Shape	Sculptural type	Puncture
Eriobotrya deflexa f. *deflexa*	25.1	26.3	0.95	Nearly spherical	Wrinkle wave and shallow bulk stripe	None
Eriobotrya deflexa f. *buisanensis*	22.3	22.8	0.98	Nearly spherical	Scattered deep stripes	Sparse
Eriobotrya deflexa f. *koshunensis*	21.2	22.0	0.96	Nearly spherical	Scattered deep stripes	Dense

表 4. 台灣原生枇杷 ITS 序列長度及其 GC 含量

Table 4. Length and GC content of ITS sequence of native *Eriobotrya* in Taiwan

Code [z]	Length of ITS1 (bp)	GC Content of ITS1 (%)	Length of ITS2 (bp)	GC Content of ITS2 (%)	Length/GC Content of 5.8 S (bp)/(%)
01D	223	64.57	204	70.59	165/65.45
02D	223	64.57	204	69.12	165/65.45
03D	223	65.02	204	70.09	165/65.45
04D	223	65.02	204	70.59	165/65.45
05D	223	65.02	204	70.59	165/65.45
06D	223	64.57	204	69.61	165/65.45
07D	223	64.57	204	70.59	165/65.45
01B	223	64.57	204	70.09	165/65.45
02B	223	64.57	204	70.09	165/65.45
03B	223	64.57	204	70.59	165/65.45
04B	223	64.57	204	70.09	165/65.45
01K	223	64.57	204	70.09	165/65.45
02K	223	64.57	204	70.09	165/65.45
03K	223	65.02	204	69.12	165/65.45

[z] Code: see Table 2.

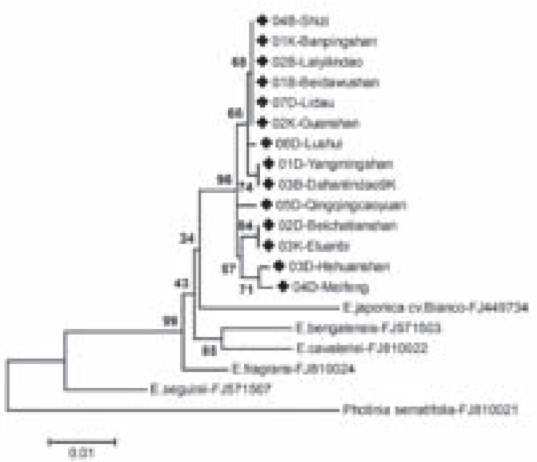

圖 5. 由 ITS 序列建構之台灣原生枇杷及枇杷之鄰接法親緣關係樹。

Fig. 5. Neighbour-joining phylogenic tree inferred from ITS data. The value of the interior branch tests > 50% is shown on each branch. Branch lengths are proportional to the number of base changes along each branch. The solid diamond (◆) indicates the native Eriobotrya in Taiwan. (Code: see Table 2).

台灣枇杷與武威山枇杷間為0.0041，台灣枇杷與恆春山枇杷間為0.0044，武威山枇杷與恆春山枇杷間為0.0022，約是台灣枇杷與武威山枇杷間及台灣枇杷與恆春山枇杷間平均遺傳距離的1/2；而台灣枇杷不同樣品間之平均遺傳距離則為0.0058，武威山枇杷為0.0009，恆春山枇杷間為0.0035。

***matK*部分序列親緣關係分析：**以前述相同方法解序14份台灣原生枇杷之*matK*部分序列並進行栽培種枇杷 (*E. japonica*) 之此部分*matK*序列 (GenBank accession number: GQ434196) 比對，共獲得658 bp，所有序列於排序後並無任何的變異位點，GC含量為34.2%。

此資料進一步以NCBI下載之栽培種枇杷與石楠為外群，以其部分*matK*序列建構鄰接親緣關係樹 (圖6)，同樣以interior branch test重複1000次評估各分枝點的可信度。結果也顯示各地採集的台灣原生枇杷樣品聚結為一群，其可信度達92%，並與其他參考材料分開。

討　　論

台灣原生枇杷屬過去常被區分為2至3個型

表 5. 台灣原生枇杷三群及群間之平均遺傳距離

Table 5. Average genetic distance among the three taxa of native *Eriobotrya* in Taiwan and among samples of each taxon (diagonal)

Taxon	*Eriobotrya deflexa* f. *deflexa*	*Eriobotrya deflexa* f. *buisanensis*	*Eriobotrya deflexa* f. *koshunensis*
E. deflexa f. *deflexa*	0.0058		
E. deflexa f. *buisanensis*	0.0041	0.0009	
E. deflexa f. *koshunensis*	0.0044	0.0022	0.0035

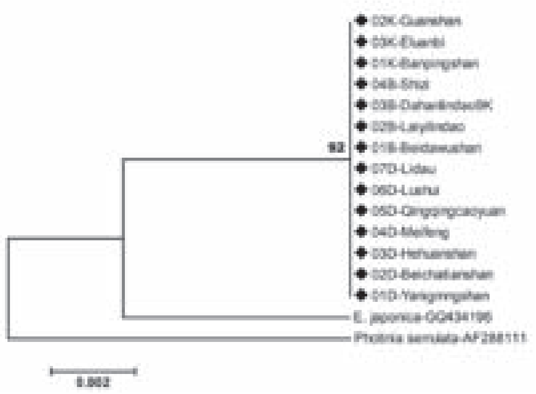

圖 6. 由 *matK* 部分序列建構之台灣原生枇杷及枇杷之鄰接法親緣關係樹。

Fig. 6. Neighbour-joining phylogenic tree inferred from partly *matK* data. The value of the interior branch tests > 50% is shown on each branch. Branch lengths are proportional to the number of base changes along each branch. The solid diamond (◆) indicates the native *Eriobotrya* in Taiwan. (Code: see Table 2).

或變種，本試驗整合形態特性、花粉形態及分子層次的資料，期望能進一步釐清其分類階級與遺傳親緣關係。

枇杷屬植物主要起源於亞洲中國，其中至少有21分類群 (taxa) 即原生於此 (Badenes *et al.* 2009; Li *et al.* 2011a; Lin *et al.* 2004; Yang *et al.* 2005, 2007b, 2007c; Yang & Lin 2007)。雖然中國大陸的枇杷屬遺傳資源研究將*E. deflexa*材料加入分析，但僅含台灣枇杷及恆春山枇杷等二份材料。本試驗之*E. deflexa*參試材料為採自台灣各地之台灣枇杷及其種下分類群樣品共14份材料，並加入原生亞洲大陸的物種參與分析。ITS序列與*matK*基因部分序列分析結果均指出台灣原生枇杷為單源群 (monophyletic group)，沒有混入參考的原生於亞洲中國的種或一些栽培種，此結果不僅支持 Li *et al.* (2007, 2009, 2011a) 的推論，並指出台灣原生枇杷在遺傳上的獨立性，顯示台灣原生枇杷應為同一種。

雖然台灣原生枇杷為單源群，但由花序之被毛情形 (圖7–9)、葉部形態 (圖10) 及花粉外壁紋飾 (表3) 可清楚地將其區分出台灣枇杷 (圖2、7)、武威山枇杷 (圖3、8) 及恆春山枇杷 (圖4、9) 等三群。武威山枇杷的分布範圍從屏東大武山以南，恆春山枇杷的分布範圍則侷限於恆春及高雄低海拔高位珊瑚礁岩區，兩間的平均遺傳距離最小 (0.0022)，雖然形態上兩者花序皆被淡褐色至灰白色短毛，但兩者的葉片與花粉粒形態可明顯地區分。武威山枇杷的葉片為圓披針形，長9–14 cm，寬1.5–2.5 cm，葉緣鋸齒狀，先端銳尖至漸尖 (圖10)，花粉外壁刺穿孔較稀疏 (圖3)；而恆春山枇杷的葉片呈倒卵長圓形，長7–14 cm，寬3–7 cm，葉緣淺圓齒狀或近全緣，先端圓鈍或鈍尖 (圖10)，花粉外壁刺穿孔較多 (圖4)。台灣枇杷廣泛分布於屏東大武山以北各地，與其他兩群間的平均遺傳距離皆為武威山枇杷與恆春山枇杷間者2倍 (0.0041, 0.0044)。形態上，台灣枇杷的花序為密被棕色長絨毛，葉長橢圓形，長10–25 cm，寬3–7 cm，葉緣鋸齒狀，先端銳尖至漸尖 (圖10)，花粉外壁紋飾為皺波散條紋較淺且無刺穿孔 (圖2)，可以清楚地與武威山枇杷及恆春山枇杷區別。

武威山枇杷與恆春山枇杷的分布與範圍大

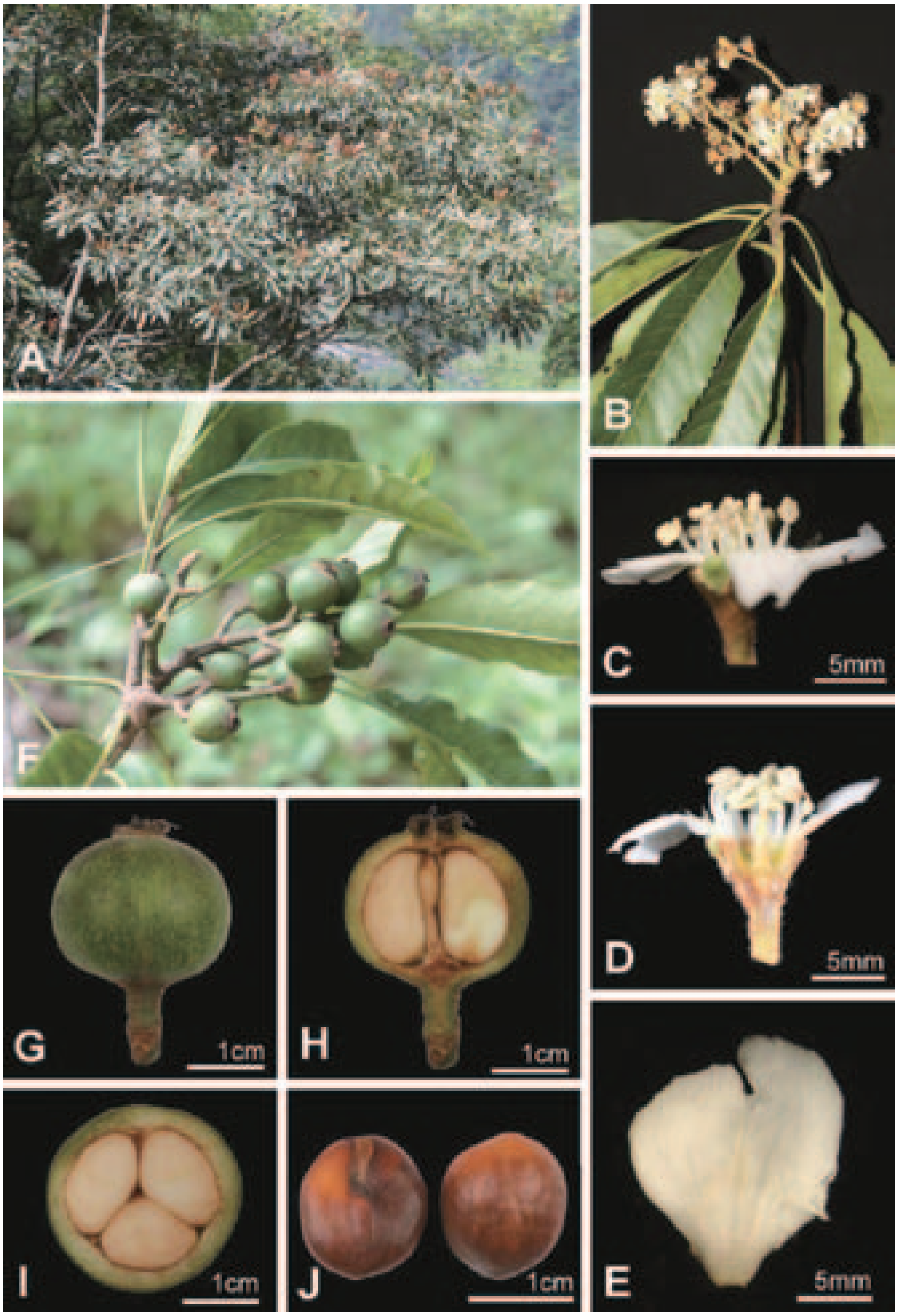

圖 7. 台灣枇杷。(A) 樹形；(B) 花序；(C) 花；(D) 花縱剖；(E) 花瓣；(F) 果序；(G) 果；(H) 果縱剖面；(I) 果橫剖面；(J) 種子。

Fig. 7. *Eriobotrya deflexa* f. *deflexa*. (A) Tree shape; (B) Inflorescence; (C) A flower; (D) Vertical-section of a flower; (E) A flower petal; (F) A fruit bunch; (G) Surface view of a fruit; (H) Vertical section of a fruit; (I) Cross-section of a fruit and (J) Seeds.

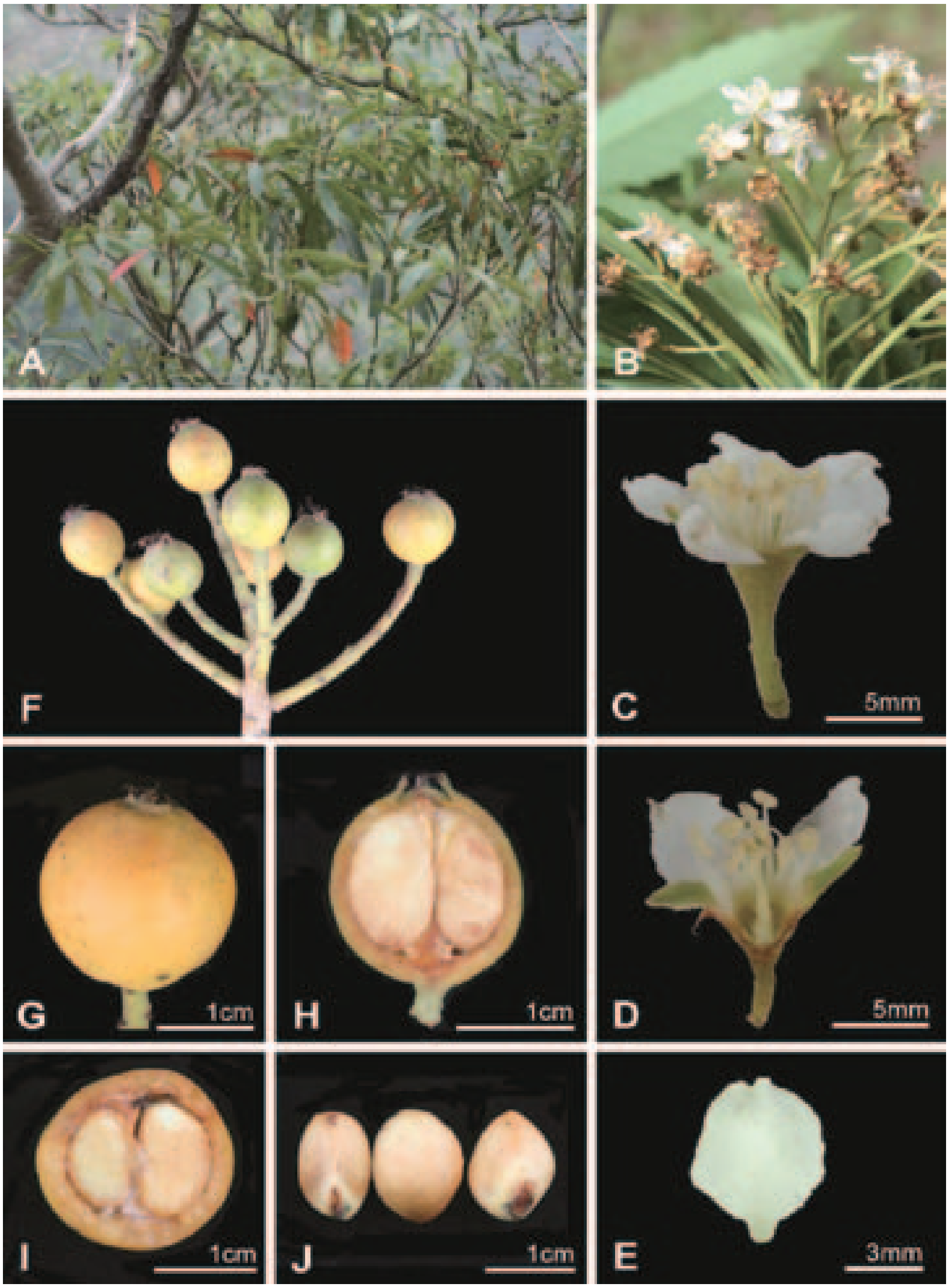

圖 8. 武威山枇杷。(A) 樹形；(B) 花序；(C) 花；(D) 花縱剖；(E) 花瓣；(F) 果序；(G) 果；(H) 果縱剖面；(I) 果橫剖面；(J) 種子。

Fig. 8. *Eriobotrya deflexa* f. *buisanensis*. (A) Tree shape; (B) Inflorescence; (C) A flower; (D) Vertical-section of a flower; (E) A flower petal; (F) A fruit bunch; (G) Surface view of a fruit; (H) Vertical section of a fruit; (I) Cross-section of a fruit and (J) Seeds.

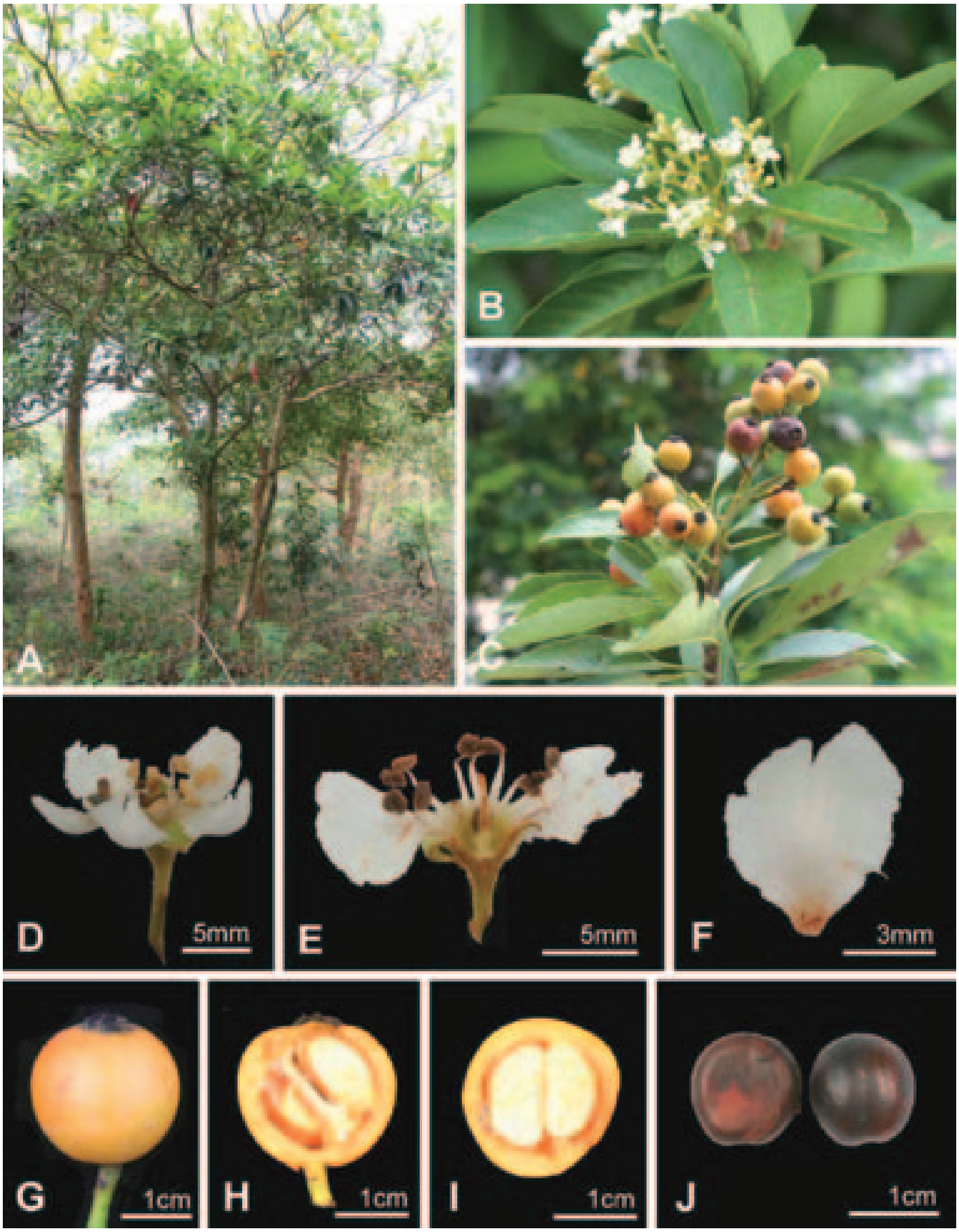

圖 9. 恆春山枇杷。(A) 樹形；(B) 花序；(C) 花；(D) 花縱剖；(E) 花瓣；(F) 果序；(G) 果；(H) 果縱剖面；(I) 果橫剖面；(J) 種子。

Fig. 9. *Eriobotrya deflexa* f. *koshunensis*. (A) Tree shape; (B) Inflorescence; (C) A flower; (D) Vertical-section of a flower; (E) A flower petal; (F) A fruit bunch; (G) Surface view of a fruit; (H) Vertical section of a fruit; (I) Cross-section of a fruit and (J) Seeds.

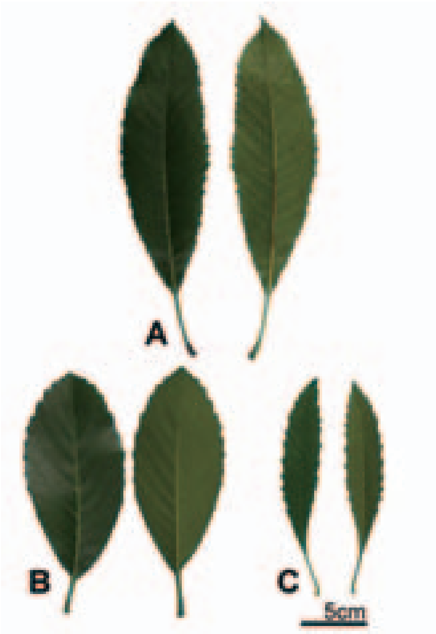

圖 10. 台灣原生枇杷分類群之葉部形態比較。(A) 台灣枇杷；(B) 恆春山枇杷；(C) 武威山枇杷。

Fig. 10. Comparision of leaf morphlogy of native *Eriobotrya* in Taiwan. (A) f. *deflexa*; (B) f. *koshunensis* and (C) f. *buisanensis*.

小相當接近，然而恆春山枇杷樣品間的平均遺傳距離卻是武威山枇杷者的4倍 (表5)，此種高度歧異化的情形值得進一步的探討；台灣枇杷分布範圍廣泛，但在大武山以南幾乎不見蹤跡，其樣品間的平均遺傳距離 (0.0058) 則分別是恆春山枇杷 (0.0035) 與武威山枇杷 (0.0009) 者的1.7與6.4倍左右 (表5)，此種區域性的分布情形是否受氣候環境 (Su *et al.* 2009; Wendel & Dolye 1998) 或其他因素所造成，則有待進一步的分析。

Lin *et al.* (2004)、Li *et al.* (2009) 及Yang & Lin (2007) 依據形態特徵及分子標誌分析的結果，將台灣原生枇杷歸屬於多雄蕊、少柱頭與葉片大小中等組 (section) 之西江亞組，其特徵為雄蕊數多於20枚、柱頭數少於 (含) 4枚。Li *et al.* (2009) 並指出台灣原生枇杷是後生種，分布範圍包括台灣、海南與廣東信宜等地。演化途徑為其原始種*E. serrate* Vidal (齒葉枇杷) 與*E. kwangsiensis* Chun (廣西枇杷) 沿著西江流域與周邊輻射散布，並發生DNA序列的交換而演化發生。惟*E. kwangsiensis* Chun至今仍未明確描述 (Lin *et al.* 2004)，而台灣原生枇杷甚至曾被稱為台廣枇杷 (Yu 1979)，在廣東鳳凰山 (Fenghuanshan) 也有採集的紀錄 (Yang & Lin 2007)，且*E. kwangsiensis* Chun的葉形為長橢圓葉，與恆春山枇杷葉形相似；又*E. serrate* Vidal至今仍難以歸類 (Yang & Lin 2007)，其葉片倒卵形或倒披針形及葉緣有鋸齒的特徵則與台灣枇杷及武威山枇杷葉形相似。因此*E. serrate* Vidal與*E. kwangsiensis* Chun是否為台灣原生枇杷的原始種仍有待釐清。

本研究整合形態性狀、花粉粒形態、核基因組ITS序列及葉綠體基因組*matK*基因部分序列之資料，確認台灣枇杷、武威山枇杷及恆春山枇杷等三群之親緣關係非常相近，且屬同一個種，雖然在葉部形態、花序之被毛情形及花粉外壁紋飾有明顯差異，但目前之分子資料仍無法有效區分種下分類群，未來將增加其他分子資料，做進一步的釐清。

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Phylogenetic and Taxonomic Study of Native *Eriobotrya* in Taiwan[1]

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Abstract

Chang, K. C., H. L. Chiu, Y. H. Tseng, and S. L. Deng. 2012. Phylogenetic and taxonomic study of native *Eriobotrya* in Taiwan. J. Taiwan Agric. Res. 61:12–28.

The native *Eriobotrya* plants in Taiwan are the wild relative of loquat crop, and they distribute across the entire island of Taiwan. The taxon of native *Eriobotrya* in Taiwan was treated inconsistently in past decades, either as species, variety, or forma. This study was conducted to reassess the systematic status of native *Eriobotrya* plants in Taiwan by investigations of the internal transcribed spacers (ITS) region sequences of nuclear ribosomal DNA (nrDNA), partly *matK* gene sequences of chloroplast DNA (cpDNA), and morphological features of leaves, inflorescences, and pollens. Results of ITS sequences analysis showed that only 7 variation sites were detected in 593 base pairs (bp) of ITS sequences obtained from the 14 samples of *Eriobotrya* plants collected in Taiwan. In addition, no variation sites were detected in 658 bp of partial *matK* gene sequences. Phylogenetic analysis showed that the native *Eriobotrya* in Taiwan belonged to one monophyletic group which was well separated from other referenced taxa from National Center for Biotechnology Information (NCBI). However, the distinction among three taxa of the native *Eriobotrya* in Taiwan was still unclear. If affirmed by more other nucleotide sequences would support to clarify the taxon under *Eriobotrya deflexa*.

Key words: *Eriobotrya deflexa*, Rosaceae, ITS sequences, *matK* sequences, Phylogeny.

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台灣農業研究 (J. Taiwan Agric. Res.) 61(1):29–37 (2012)

農業研究人員對產學合作計畫之參與動機與滿意度研究[1]

楊舜臣[2,3]

摘　要

楊舜臣。2012。農業研究人員對產學合作計畫之參與動機與滿意度研究。台灣農業研究 61:29–37。

產學合作係指學術界或研究單位和產業界間共同合作、研發，使理論與實務能有效整合之合作策略，進而達到研究、資源的有效利用及人才培育等之意涵。本研究針對行政院農業委員會所屬試驗改良場所曾進行農業科技產學合作計畫研提人進行問卷調查，調查結果顯示：受訪者對於參與產學合作計畫動機之認同度最高之前三名依序為「研究單位政策」、「瞭解產業需求，開發創新研究主題」及「解決業者研發瓶頸」等，比較偏重政策導向與產業導向，另對於聲望導向與績效導向等，並不是迫切合作之動機。另在滿意度調查方面，受訪者對於農業科技產學合作計畫制度多保持正面的回應 (有39.2%的受訪者表示滿意或非常滿意)，但仍有16.4%的受訪者表示不滿意或非常不滿意，並提出改善建議。

關鍵詞：產學合作、創新機制、參與動機、滿意度。

前　言

產學合作係指學術界或研究單位和產業界間共同合作、研發，使理論與實務能有效整合之合作策略，進而達到研究、資源的有效利用及人才培育等之意涵。為有效提升產業、國家的競爭力，國內推動產學合作的單位是以經濟部、國家科學委員會、教育部及行政院農業委員會為主。目前經濟部、國科會、教育部及農委會各有專職負責推動產學合作體系之窗口：經濟部的創新育成中心，負責輔導中小企業的創新培育；國科會的技術移轉中心，負責輔導專利管理、智慧財產權的維護及商品化的技術移轉；教育部的區域產學合作中心，專職區域產學合作的推廣業務與媒合，另外技職專校設有40個專屬領域的技術研發中心，負責技術的創新與研發；農委會的科技處，負責研發成果管理、運用及產學合作計畫之規劃與執行。

Li (2003) 進行我國產學合作的影響因素之實證研究，有關產學合作動機及其影響因素方面：(1) 具有產學合作經驗的大學教授對於產學合作動機具有較高的認同程度；(2) 學校屬性與「參與實務研究的機會」的產學合作動機有相關性；(3) 大學教授職等與「提高研究水準與學術聲望」的產學合作動機有相關性；

1. 行政院農業委員會農業試驗所研究報告第2618號。接受日期：101年1月6日。
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(4) 大學教授的教學年資與「增加學生的實務經驗」的產學合作動機有關連性；(5) 具有產學合作的企業研發經理人對產學合作動機具有較低的認同程度；(6) 公司員工人數的多寡與「為了解決公司的特殊問題」、「使用學校特殊的設備」的產學合作動機有相關性。

Huang (2010) 彙總各學者對於產學合作雙方參與動機，其中有關學研單位動機方面分析如下：(1) 基於市場導向，如擬將學術研究加以應用/商品化、接觸較前端之研究領域或技術知識、借重企業人員商業經驗、增進理論與實務的結合、吸取企業管理經驗及獲得產業/市場的資訊等；(2) 基於資源導向，如擬增加財務來源、使用企業的設備與原料、激發內部研發創意、企求更好的績效考核、增加服務單位良好形象及聲望等。而其研究發現，研究人員參與產學合作之動機對合作績效滿意度呈顯著之正向影響，且合作動機透過良好溝通及加上較高制度滿意度對合作績效滿意度亦呈顯著之正向影響。

Wu (2005) 進行大學教師參與產學合作之行為意向模式研究發現：大學教師之「態度」構面及「知覺行為控制」構面對於參與產學合作之行為意向呈正相關。而經由結構方程模式分析，說明「自利性」及「利社會性」對於「態度」構面，具有正向影響關係；而「知覺行為控制」構面中，「自我效能」及「便利狀態」二因素具有正向影響關係。

Chiu (2005) 進行產學合作計畫績效之研究發現：(1) 在資源依賴程度方面，產學在合作計畫中，計畫形成的關鍵在於大學教授有良好的知識、技術可以解決問題或開發成為產品；(2) 在結構面方面，產學合作關係中雙方人員有直接的溝通管道且互動層級越高，便於達成共識且即時解決問題；(3) 在關係面方面，產學合作間良好的互動可增進對彼此的信任、義務及認同感，進而影響合作績效；(4) 在認知面方面，產學雙方對於合作計畫的內容瞭解越清楚且預期目標明確，在合作的進行會較為順暢且合作成果也能夠讓雙方都滿意。

行政院農業委員會於1998年8月公告實施「行政院農業委員會農業科技計畫產學合作實施要點」，對於農業科技產學合作計畫定義為：(1) 以本會或所屬機關農業科技計畫研發已有初步成果，擬商品化者為合作計畫項目；(2) 以企業研發技術已有初步成果，擬商品化者為合作項目，但不包括委託檢驗、鑑定、測試或試驗等項目。本要點實施以來成功技轉率達3成以上，頗具成效，惟本制度執行至今已有一段時間，研究同仁對於參與產學合作計畫之參與動機與是否滿意，則是本研究之探討重點，並藉以瞭解本制度是否仍有改進空間。

材料與方法

問卷設計

依據研究目的設計問卷，建立代表性題目，著重於「參與動機」與「滿意度」二大區塊。在參與動機方面，以政策、經費、聲望、績效、產業、資源等6大導向，設計問卷題目共14題；在對制度滿意度方面，以機制、經費、年限、績效、互動等面向，設計問卷題目共17題；每個題目均提供開放式填答空間，彌補封閉式問卷之不足，以探知研究人員實際之反應。

本問卷採用李克特五點量表計分方式，在參與動機方面包含「非常同意」、「很同意」、「普通」、「不同意」、「非常不同意」五個等級，而在滿意度方面則涵括「非常滿意」、「很滿意」、「無意見」、「不滿意」、「非常不滿意」五個等級，分別給予5至1分的評定。

研究對象

本研究以曾執行行政院農業委員會農業科技產學合作計畫之研究人員為調查對象，依據行政院農業委員會科技處的資料，從2004年起至2010年止，共計有499位研究人員曾研提過

農業科技產學合作計畫。

資料蒐集方法

本研究採用問卷調查為資料蒐集方法，問卷於2010年10月28日寄出，由各試驗改良場所研發成果管理人員協助送交該單位曾執行農業科技產學合作計畫之研究人員，並訂於同年11月15日為問卷回收截止日。在回收截止日後，經多次稽催，總計回收159份問卷，除林業試驗所外，其餘各單位均有問卷回收，有效問卷回收率為31.9%。

資料分析方法

本研究以SPSS統計套裝軟體進行資料分析，所使用到的統計方法為次數分配、百分比、平均值、標準差 (standard deviation, SD) 及單因子變異數分析 (one-way ANOVA)。

信度分析

在信度考驗部分，本問卷採用Cronbach's α信度係數以進行內部一致性考驗，分析各題目及整體施測的穩定性，根據Nunnally (1978) 的觀點，Cronbach's α值至少要大於0.5，最好能大於0.7，Cronbach's α值越大表示題目間一致性越高，亦即代表問卷的信度越好。本問卷全部分析項目的Cronbach's α值為0.856，可見本研究之量表具高信度，為可行的研究工具。

結　　果

受訪者基本資料

本次問卷回收情形如表1所示，受訪者服務單位分布情形，以區農業改良場最高 (District Agricultural Research and Extension)，7個改良場共占49.1%，其次是試驗所 (Research Institute)，6個試驗所共占35.2%；其餘3個專業場共占15.7%。

另受訪者專長領域分布情形，以農業技術最高，占64.8%；受訪者性別分布情形，以男性占76.7%為最高，女性占23.3%；受訪者教育程度分布情形，以碩士占57.9%為最高，其次是博士占35.2%；受訪者年齡分布情形，以

表 1. 問卷回收情形
Table 1. Number of researchers participated in the survey and responded rate at various research institutes in Taiwan

Institute	No. researchers received questionaire	Percentage (%)
Taiwan Agricultural Research Institute	24	15.09
Taiwan Forestry Research Institute	0	0
Fisheries Research Institute	7	4.40
Livestock Research Institute	13	8.18
Animal Health Research Institute	6	3.77
Taiwan Agricultural Chemicals and Toxic Substances Research Institute	6	3.77
Taoyuan District Agricultural Research and Extension Station	14	8.81
Miaoli District Agricultural Research and Extension Station	6	3.77
Taichung District Agricultural Research and Extension Station	12	7.55
Tainan District Agricultural Research and Extension Station	14	8.81
Kaohsiung District Agricultural Research and Extension Station	14	8.81
Hualien District Agricultural Research and Extension Station	13	8.18
Taitung District Agricultural Research and Extension Station	5	3.14
Endemic Species Research Institute	7	4.40
Taiwan Seed Improvement and Propagation Station	8	5.03
Tea Research and Extension Station	10	6.29
Total	159	100

10歲為區間，41–50歲占44.7%為最高，其次是51–60歲占35.8%；受訪者職稱分布情形，以副研究員級占43.3%為最高，其次是助理研究員級占35%。

受訪者對農業科技產學合作計畫之參與動機分析

受訪者對於參與產學合作計畫動機之認同度分析結果如表2所示，其中認同度平均數最高之前三名依序為「研究單位政策」、「瞭解產業需求，開發創新研究主題」及「解決業者研發瓶頸」等，偏重政策導向與產業導向。在所有動機問卷中，以「研究單位政策」問項之高低分差距最小 (標準差為0.62)，顯示受訪者對於「研究單位政策」之參與認同度較為一致；但「人力經費支援」問項之高低分差距較大 (標準差為1.08)，顯示受訪者對於因考量「人力經費支援」而參與的認同度差異較大。

受訪者對農業科技產學合作計畫制度之滿意度分析

受訪者對於產學合作計畫制度之整體滿意程度分析結果如圖1所示，有39.2%的受訪者表示滿意或非常滿意，但仍有16.4%的受訪者表示不滿意或非常不滿意。

受訪者對於計畫經費及合作制度之滿意度情形，如表3所示。在計畫經費面向，合計41.7%的受訪者滿意及非常滿意99–102年農業科技產學合作計畫經費由行政院農業委員會以優先推動計畫支援 (表3)，但仍有其他否定看法，包括：(1) 評審方式與機制未能符合地區

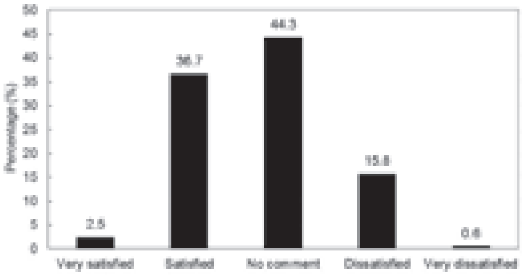

圖 1. 受訪者對於整體產學合作計畫機制之滿意程度。

Fig. 1. Survey of agricultural researchers in COA for level of satisfaction of participating in Industry-University Cooperation projects.

表 2. 受訪者對於參與產學合作計畫之參與動機分析

Table 2. Analysis of researchers' motivation for participation in Industry-University Cooperation projects

Item	Participant's Motive	Mean	SD	Rank
Policy	Policy of research institute	3.96	0.62 [z]	1
Funding	Funding for personnel	2.96	1.08	13
	Funding for equipment	3.12	0.98	12
	Funding for research	3.58	0.84	4
Reputation	Increase R&D capability	3.54	0.90	6
	Raise research reputation	3.36	0.94	9
Efficacy	Profit from technology transfer	2.93	0.93	14
	Personal performance	3.16	0.95	11
Industry	Participators submit cooperation requires	3.45	0.91	7
	Aware of industrial needs and develop innovative research subjects	3.81	0.72	2
	Help industry solving problems encountered in research	3.74	0.83	3
Resource	Reliance on industrial equipments and experiences	3.21	0.95	10
	Test run for mass production of research product	3.58	0.81	5
	Shorten the time needed for research and technology transfer	3.45	0.88	8

[z] Respondents for the 'Policy of research institute' is more consistent.

表 3. 受訪者對於計畫經費與合作機制之滿意度情形
Table 3. Analysis of researchers' satisfaction on funding and cooperation of Industry-University Cooperation projects

		Satisfaction (%)				
Item	Question	Very satisfied	Satisfied	No comment	Dissatisfied	Very dissatisfied
Project funding	Current special funding	7.7	34.0	54.5	3.8	—
	Adjust institute's fund supported industry-university cooperation projects in future	1.9	19.7	44.6	24.2	9.6
	Proportion of investment by the industry	1.9	41.0	53.2	—	—
Cooperation set-up	Unified project reviewing	1.3	23.7	55.8	16.7	2.6
	Application procedure and time	0.6	28.8	64.1	6.4	—
	Industry's eligibility	1.9	38.2	57.3	2.5	—
	Two year contract limit	—	33.8	54.8	11.5	—

性產學合作發展需求；(2) 以產值評估優先順序，小產業生存空間易受壓縮，建議評估標準應以本土性特色化為考量。有33.8%的受訪者不滿意及非常不滿意未來再由各單位預算提撥10%做為農業科技產學合作計畫之經費 (表3)，而建議應由農業部編列預算以專款支應，因各單位經費逐年減少，提撥10%會壓縮與排擠一般科技計畫經費等。有42.9%的受訪者對於目前農業科技產學合作計畫業者出資比例持滿意及非常滿意的正向看法 (表3)，其他認為不滿意的看法則指出出資比例太低。有61.6%受訪者認為經費編列並無受到限制，但認為有受限者指出人力與設備受勞務工總量管制及年度資本門分配所限而無法編列、以現有計畫人力支援對其他計畫產生排擠效應、購置之產業化設備設限於財產無法移轉等，並提出產學合作計畫能比照國科會編列計畫助理方式、產業化設備能於技術移轉時併入授權標的內等建議。有86.3%的受訪者認同所編列之農業科技產學合作計畫經費能完全支應計畫執行，但少數無法完全支應計畫執行之原因，為原提計畫將部分經費提撥為水電費與統籌勞務外包用、經費預算並未專款專用或由於刪減成數太高，致無法達成預定目標等。

在合作機制面向之分析結果如表3所示，有25%的受訪者滿意及非常滿意目前農業科技產學合作計畫統一審查方式，但仍有19.3%不滿意及非常不滿意，其否定看法與建議為：(1) 書面審查無法顯示各計畫重複性，且有區域發展重點，應讓研究人員報告計畫構想；(2) 審查委員對於整體或區域產業未必相當瞭解，應以業界需求為主；(3) 產業差異度大，統一審查毫無意義；(4) 部分委員非該領域專長，有時所提意見實務上無法執行；(5) 各單位提出產學合作計畫係依轄區產業發展需求為優先考量，現有評審機制並無法充分考量轄區發展，建議回歸單位內審等。僅有29.4%的受訪者滿意及非常滿意目前農業科技產學合作計畫之申請程序與時程，不滿意者認為目前的程序繁複、時程太慢及無法掌握先機，應予簡化並隨到隨審，以因應業界的快速變化。有40.1%的受訪者滿意及非常滿意目前農業科技產學合作計畫申請業者之資格條件，少數不同看法認為應放寬至農民、產銷班及飼養業者等 (非以法人為限)。33.8%的受訪者滿意目前農業科技產學合作計畫以2年為限之政策，不滿意者則認為2–3年較1–2年佳，並應依計畫性質給予不同考量，必要時得以延長。另對於產學合作計畫與技術移轉整體流程方面，受訪者提出委託合適單位統

一辦理招商作業，並對單位內之行政部門加強相關法規之教育訓練等建議。

受訪者對於績效考評之滿意度情形如圖2所示，27.6%認為成效考核採技術移轉成功率計算是滿意與非常滿意，但仍有28.2%認為不滿意與非常不滿意，其否定之看法主要為：(1) 研究結果非預期、受限法規及登記程序等，以致業者承接意願低；(2) 技術層次高低有差，且可能無法立即技轉，後續之研發改進仍有技轉之可能等。

受訪者對計畫執行成效之看法分析

受訪者執行產學合作計畫後，僅有47.4%的受訪者有順利技術移轉，沒有順利技術移轉之原因，主要是「技術移轉費用太高，業者不願承接」最高，比例占27.5%，其他訴諸原因，尚包括：「計畫尚在執行中」、「技術移轉尚在洽談中」、「計畫終止」、「業者需增加投資成本，包括人力、設備、模具等」、「業者財務、營運狀況不佳或轉型，停止開發」、「技術未成熟、市場非預期、利潤有限等」、「環保產品需政策之配合與協助」、「技術授權條件因智審會調整，業者不願承接」等。

在合作溝通與模式方面，73.4%的受訪者認為業者需派員參與農業科技產學合作計畫，而僅有26.6%認為不需要，並建議應由業者視需求自行決定是否派員參與，也擔心研發成果核心與關鍵技術外流，而希望能加強保密並保護研發資訊及材料，另有些業者則指出人力不足故無法派員。在農業科技產學合作計畫期間，高達98.7%的受訪者常與合作業者保持聯繫，其中以每個月聯繫1次最高，占41.3%，半年以上才聯繫甚少，僅占2% (圖3)；聯繫方式則以電話聯繫方式最高，占38% (圖4)。

96.9%的受訪者表示與業者合作間保持友善關係，有25.7%的受訪者表示業者會提出計畫以外之合作項目；對於業者提出的要求，以

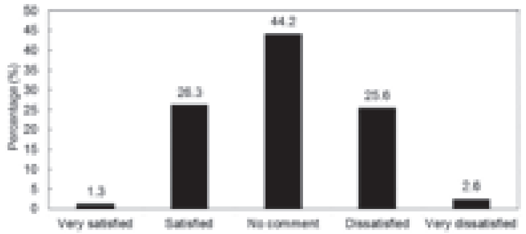

圖 2. 受訪者對於採用技轉成功率為考核指標之看法。

Fig. 2. Researchers' view on using 'success rate of technology transfer' as a yardstick for evaluation of Industry-University Cooperation projects.

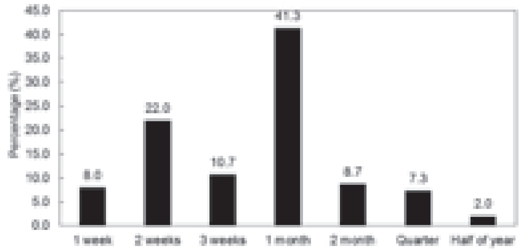

圖 3. 受訪者與合作業者聯繫之頻率。

Fig. 3. Survey of agricultural researchers in COA for level of satisfaction of participating in Industry-University Cooperation projects.

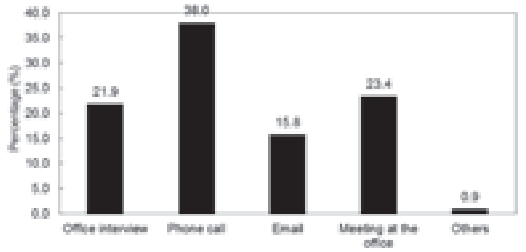

圖 4. 受訪者與合作業者聯繫方式。

Fig. 4. Analysis of methods of contact between researchers and industrial people during the period of participating in Industry-University Cooperation projects.

增加試驗規模要求最高，占67.7%；對於業者提出要求之回應，絕大多數 (占80%) 均表示樂意配合。

高達96.1%的受訪者認為目前農業科技產學合作計畫方式對業者有幫助，極少數認為由

上級統一審查，並無法切實瞭解廠商及市場需求，故對合作業者沒有幫助。而大多數受訪者也認為目前方式對自己有幫助 (占86.3%)，其他認為沒有幫助者指出：(1) 目前方式無法申請獲得適當的計畫；(2) 本身能力設備即可完成，毋須拜託廠商參與合作計畫；(3) 技術層級不高，廠商可能勝任，不需透過產學合作；(4) 單位內採購限制太多，無法符合產學合作精神；(5) 合作壓力較大等。

受訪者基本資料之單因子變異數分析 (one-way ANOVA)

本研究將受訪者基本資料 (包括年齡、教育程度、服務單位、職稱、專長領域等)，與「參與動機」問項及「滿意度」問項進行ANOVA分析，其中「職稱」與「順利技轉」、「參與動機之單位政策」、「參與動機之提升研發能力」、「借重合作業者的設備與經驗」及「產學經費支援」等問項，達顯著水準，經事後比較考驗，在「順利技轉」問項，助理研究員較研究員能順利技轉；在「參與動機之單位政策」問項，副研究員認同度較助理研究員高；在「產學經費支援」問項，副研究員較研究員滿意。

另在「服務單位」與「個人績效考量」、「業者主動提出合作需求」等問項，達顯著水準，經事後比較考驗，在「個人績效考量」問項，專業場研究人員較區改良場來得重視；在「業者主動提出合作需求」問項，試驗所研究人員較區改良場與專業場來的低，顯示區改良場與專業場較能與產業互動，所提產學合作計畫較符合產業需求。

受訪者對農業科技產學合作計畫制度之其他建議

對產學合作計畫標的與對象之建議：(1) 產學合作之範圍應可比照學術機關擴及至委託試驗、技術諮詢等；(2) 產學合作業者應具後續商品化之能力，應落實業者資格審核機制；(3) 突破與學術單位跨機關間合作機制，充分運用現有資源，提升研發能力。

在產學合作計畫後續追蹤上之建議：(1) 應積極追蹤產學合作計畫結束後之成效，對於後續未能順利轉移者，應瞭解其技術是否已外流；(2) 產學合作應搭配先期技轉，避免合作過程中技術被業者探知，俟計畫結束後已無承接意願；(3) 應有統一窗口整合其他部門資源，協助解決產業化過程之瓶頸及障礙；(4) 部分產學合作計畫為解決區域性農業問題且為推廣服務，計畫結束後如能改善轄區農民問題，應可為技轉外之另一績效評核指標；(5) 產學合作件數不宜列入單位績效評核指標，才不致濫竽充數。

關於產學合作成果技轉移轉之建議：(1) 產學合作計畫後之技術移轉，宜考量業者再投入成本與市場性，酌予調整授權金額度；而應以未來銷售情形回饋衍生利益金較為合理；(2) 應先考量技術移轉之標的在未來是否可再展延，並應於契約內註明，以避免授權期限屆期之困擾。

而落實產學合作之建議：產學合作計畫之目的在於使研究成果產業化，各單位宜鼓勵研究人員參與，但因各場所對此重視程度不一，建議納入獎勵機制，以獎勵研究人員參與並加速落實研發成果產業化應用。

結　　論

本研究結果顯示，受訪者對於參與產學合作計畫動機之認同度最高之前三名依序為「研究單位政策」、「瞭解產業需求，開發創新研究主題」及「解決業者研發瓶頸」等，較偏重於政策導向與產業導向，另對於聲望導向與績效導向等，並不是迫切合作之動機。受訪者對於農業科技產學合作計畫制度多保持正面的回

應 (39.2%表示滿意或非常滿意)，但仍有16.4%不滿意或非常不滿意，其認為未來應予改善處，包括：(1) 產學合作計畫範圍、資格條件與計畫年限之鬆綁；(2) 區域性產業之產學合作計畫審查回歸各單位自行審查；(3) 簡化計畫流程與技術移轉程序，並由統一窗口辦理公開徵求活動等；(4) 產學合作經費應由農委會編列，不應由各單位預算提撥，避免壓縮其他科技計畫之經費；產學合作經費應專款專用；支援研究人力與購置設備能更具彈性；(5) 產學合作之績效不能單以技轉率來進行考核，單位內之績效評估亦不應以產學合作計畫件數與經費來評核，沒有透過技術移轉而能對產業或農民有幫助者，亦可達到落實產業應用之目的；(6) 技術移轉的授權金應考量產業規模、公司再投入之成本，不應盲目追求授權金金額，應思考如何對產業最有助益。

誌　　謝

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Study on Motivation and Satisfaction of Agricultural Researchers' Participation in Industry-University Cooperation Projects[1]

Shen-Chen Yang[2,3]

Abstract

Yang, S. C. 2012. Study on motivation and satisfaction of agricultural researchers' participation in industry-university cooperation projects. J. Taiwan Agric. Res. 61:29–37.

The term of Industry-University Cooperation is defined as a joint effort on research and development (R & D) between a research institute and an industry for achieving a common goal of interest to both parties. This study was to conduct a survey on agricultural researchers, affiliated with Council of Agriculture (COA) and with experience in participating in Industry-University Cooperation projects, for understanding their motivation and satisfaction of participation in this type of projects. Results showed that researchers' motivation to participate in Industry-University Cooperation projects was determined most importantly by 'the policy of his/her research institute,' followed by 'the effort to develop innovative research, according to the needs of the industry' and then 'the effort to over-come bottle necks of the project encountered by the industry.' The survey also showed that earning reputation and recognition from participating in this type of projects was not a major interest for researchers. Results of this survey also showed that 39.2% of the researchers survied were satisfied or very satisfied with their participation in Industry-University Cooperation projects, whereas 16.4% of the researchers were dissatisfied or very dissatisfied.

Key words: Industry-university cooperation, Innovation, Motivation, Satisfaction.

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台灣地區蔬菜鎘、鉛濃度調查[1]

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張繼中[9] 蔡淑珍[2] 黃維廷[2]

摘　　要

林毓雯、劉滄棽、陳吉村、湯雪溶、陳鴻堂、卓家榮、蔡正賢、林永鴻、張繼中、蔡淑珍、黃維廷。2012。台灣地區蔬菜鎘、鉛濃度調查。台灣農業研究 61:38–51。

本研究之目的在瞭解台灣地區蔬菜鎘、鉛濃度。蔬菜樣本採自台灣地區主要蔬菜產區，共1900個樣本，取可食用部位以濃硝酸及過氯酸消化後，以感應耦合電漿原子放射光譜儀測定鎘、鉛濃度。結果顯示：各類蔬菜平均鎘濃度 (濕基) 分別為葉菜類ND–0.151 mg kg^{-1}、(半) 結球菜類ND–0.080 mg kg^{-1}、根莖菜類ND–0.116 mg kg^{-1}、瓜菜及果菜類ND–0.115 mg kg^{-1}；鉛濃度 (濕基) 則為葉菜類ND–0.294 mg kg^{-1}、(半) 結球菜類ND–0.239 mg kg^{-1}、根莖菜類ND–0.619 mg kg^{-1}、瓜菜及果菜類ND–0.064 mg kg^{-1}。蔬菜樣本中鎘濃度超出衛生署公告的「蔬果植物類重金屬限量標準」者占0.6%，鉛濃度超出限值者占0.7%，其中高麗菜、(半) 結球萵苣、胡蘿蔔分別有6.1%、9.8%、0.6%樣本鎘濃度超出限值，蘿蔔及胡蘿蔔分別有0.3%及4.0%樣本鉛濃度超出限值。

關鍵詞：鎘、鉛、蔬菜作物、食品安全。

前　　言

隨著工業發展，各種人為污染物質經由環境而進入食物鏈，使得國人健康受到威脅，其中又以重金屬污染最受關注。行政院環保署自民國72年起著手進行土壤污染調查工作，截至100年7月公告重金屬污染控制場址共200 ha，其中農地占82 ha。依據土壤及地下水污染整治法，這些場址在整治完成前禁止栽種作物，流

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入市場的機率不大。然而，台灣地狹人稠，除了主要農業區之農田外，都會區及其周邊仍有許多零星農田分布，人類活動對農田土壤或作物造成的影響有其潛在風險 (Kachenko & Singh 2006; Nabulo *et al*. 2006; Uzu *et al*. 2010)。

Chaney (1980) 曾研究污泥所含重金屬流入至食物鏈後，提出「土壤–植物障礙」(soilplant barrier) 論述。其中鎘屬於對動物或人類健康危害風險最大的第4群，本群元素對動物或人類毒害的濃度低於作物，故在作物還能正常生長的濃度下，便可能造成動物或人類健康的危害。鉛雖屬於影響動物及人類健康風險較小的第2群，它們在土體牢固的被土壤膠體所吸附，如果被作物所吸收，也將存在根部而不會立即轉運至食用部位，然而，根據研究顯示 (Uzu *et al*. 2010) 其可經由植物葉面吸收，且會危害神經系統，影響幼童智力發展 (Carrington & Bolger 1992)，為毒性極高之元素。因此，歐美等先進國家均對作物及食品訂有鎘、鉛濃度限值。我國行政院衛生署亦訂有食米、食用菇類、蔬果植物類之重金屬限量標準，其中100年5月修訂公告的「蔬果植物類重金屬限量標準」，亦規範各類蔬果作物中鎘、鉛濃度 (Department of Health, Executive Yuan 2011)。該標準係衛生署依據蔬菜食用部位、國人食用頻率，並參考歐盟限量標準後訂定。

蔬菜為國人日常飲食不可或缺的重要食物來源，依據行政院衛生署新版飲食指南 (100年7月公布) 與舊版對照，除了降低五穀根莖類及油脂的建議量，同時提高蔬菜水果的建議量，每人每日蔬菜攝取量由舊版的3份調整為3到5份，因此，蔬菜作物的安全性不容忽視。國內過去對蔬菜重金屬濃度調查資料極少 (Lin *et al*. 1992; Shih *et al*. 2008)，全省性調查資料僅 Lin *et al*. (1992) 之研究，可惜發表資料係以蔬菜類別進行統計，缺乏個別蔬菜之數據。

台灣地區因地質條件、氣候環境多樣，致使土壤性質複雜，部分地區受天然條件影響，土壤的重金屬濃度原本較高 (Liu *et al*. 2007)，且重金屬元素由土壤經由植物吸收並轉運至可食部位的多寡，受到土壤及氣候因子、植物特性及農業管理等因素所影響，為瞭解台灣地區生產蔬菜的重金屬濃度範圍，自97年起，農業試驗所與7個地區改良場合作進行台灣地區蔬菜重金屬濃度調查。

材料與方法

蔬菜樣本來源

97年至99年6月，農業試驗所與7個地區改良場共收集全台灣主要蔬菜產區1900個蔬菜樣本，採樣地點分布如圖1。所採蔬菜參考行政院衛生署100年5月30日署授食字第1001301183號令訂定「蔬果植物類重金屬限量標準」附表之蔬果分類表分類，包括：小葉菜類897件、(半)

圖 1. 台灣各縣市採樣點分布位置 (☆)。
Fig. 1. A map of Taiwan showing locations (☆) of vegetable samples collected for this study.

結球菜類111件、根莖菜類 (不含鱗莖類) 752件、瓜菜及果菜類140件。

蔬菜樣本製備與分析

每個樣本取食用部位約500 g，清洗晾乾後裝入乾淨的紙袋，以70℃烘乾。烘乾之植體以陶瓷刀具切碎後，使用純鈦製磨粉機粉碎，置於乾燥器中儲存備用。樣品以二酸混合液 (硝酸：過氯酸 = 5：1，v/v) 分解，分解及定量程序如 Lin *et al*. (2003) 所採用方法，濾液以感應耦合電漿原子放射光譜儀 (HORIBA JOBIN-YVON ULTIMA 2C) 測定鎘、鉛濃度，將測定值導入標準曲線並加計分解過程稀釋倍數，求得各樣本之鎘、鉛濃度 (乾基計)。

儀器偵測極限與方法偵測極限

以感應耦合電漿原子放射光譜儀分析空白標準液7次，計算測定值之標準偏差，以3倍標準偏差值導入標準曲線公式，計算得儀器偵測極限。準備鎘、鉛濃度為儀器偵測極限5倍濃度內之樣品，秤取7重複，依上述樣品分解及測定方法操作，測定值導入標準曲線並加計分解過程稀釋倍數，求得各重複樣品之鎘、鉛濃度，計算其標準偏差，以3倍標準偏差值為方法偵測極限。

標準參考物質分析

以購自美國國家標準和科技機構 (National Institute Standards and Technology, NIST) 之蘋果葉 (1515)、菠菜葉 (1570a)、番茄葉 (1573a) 為標準參考物質，分析方法如樣品分解及測定方法。

結　果

偵測極限與標準參考物質分析

以感應耦合電漿原子放射光譜儀分析鎘、鉛之儀器偵測極限分別為0.0004、0.0038 mg L^{-1}，方法偵測極限分別為0.026及0.182 mg kg^{-1} (乾基計)。標準參考物質分析結果詳如表1，其中菠菜葉及番茄葉鎘回收率分別為87.5%及82.9%，蘋果葉鉛回收率為113%。

台灣地區蔬菜鉛、鎘濃度

本研究之蔬菜樣本重金屬濃度分析係以乾燥樣品進行，為便於與衛生署公告之「蔬果植物類重金屬限量標準」進行比對，故分別引用美國農部 (USDA) 營養資料庫及衛生署出版之「台灣地區食品營養成分資料庫」中水分含量資料，將分析結果換算為以濕基計之濃度，方法偵測極限亦依個別作物水分含量換算為濕基，結果如表2及表3所示。

蔬菜食用部位鎘濃度範圍，小葉菜類為ND–0.151 mg kg^{-1}，(半) 結球菜類為ND–0.080 mg kg^{-1}，根莖菜類 (不含鱗莖類) 為ND–0.116 mg kg^{-1}，瓜菜及果菜類為ND–0.115 mg kg^{-1} (表2)。其中小葉菜類、瓜菜及果菜類之鎘濃度均在「蔬果植物類重金屬限量標準」之限值以內，根莖菜類(不含鱗莖類) 之胡蘿蔔有0.6%樣本鎘濃度超出限值，(半) 結球菜類之高麗菜、(半) 結球萵苣則分別有6.1%、9.8%樣本鎘濃度超出限值。若以全部蔬菜數量計，鎘濃度超出限值者占0.6%。蔬菜食用部位鉛濃度範圍，小葉菜類為ND–

表 1. 已認證參考樣品 (蘋果、菠菜及番茄葉) 之鎘、鉛測定濃度比對

Table 1. The comparison of concentration of Cd and Pb of certified reference materials (leaves of apple, spinach and tomato) determined between this laboratory and reported data [z]

	Apple leaves (NIST 1515)			Spinach leaves (NIST 1570a)			Tomato leaves (NIST 1573a)		
	Certified	Measured	Rec. (%)	Certified	Measured	Rec. (%)	Certified	Measured	Rec. (%)
Cd	0.013 ± 0.002	< 0.026	—	2.89 ± 0.07	2.53 ± 0.04	88	1.52 ± 0.04	1.26 ± 0.02	83
Pb	0.470 ± 0.024	0.533 ± 0.067	113	0.20 [y]	< 0.182	—	—	0.54 ± 0.12	—

[z] μg g^{-1} on dry weight basis.

[y] Information value (non-certified value).

表 2. 分析用蔬菜種類、樣品數、水分、鎘濃度 (包括最大、最小、平均及標準偏差值) 及超過國家標準值比率

Table 2. The species, sample size, water content, Cd concentration (values of maximum, minimum, mean and standard deviation), and rates of Cd concentration higher than the regulation levels in Taiwan

Vegetable group	Vegetable name	*n*	Moisture [z] (%)	Min. (mg kg^{-1} fresh weight)	Max.	Mean	SD [y]	Above the national standard (%)
Leaf vegetables	Pai-tsai	121	95.7	ND	0.115	0.023	0.018	0
	Spinach	18	93.0	0.048	0.133	0.079	0.022	0
	Mustard	86	94.6	ND	0.053	0.019	0.012	0
	Leafy lettuce	77	96.9	ND	0.073	0.019	0.016	0
	Water spinach	124	92.8	0.005	0.132	0.022	0.022	0
	Amaranth	25	93.9	0.008	0.050	0.021	0.009	0
	Pak-choi	101	94.8	ND	0.096	0.022	0.015	0
	Rape	24	95.4	0.010	0.044	0.023	0.009	0
	Velvet plant	31	92.6	ND	0.146	0.033	0.035	0
	Ceylon spinach	16	93.1	0.005	0.055	0.027	0.014	0
	Garlandchry santhemum	20	95.0	ND	0.087	0.031	0.021	0
	Celery	26	94.7	0.011	0.151	0.037	0.026	0
	Green onion	228	92.2	ND	0.146	0.023	0.023	0
Head vegetables	Cabbage	49	93.5	ND	0.061	0.016	0.016	6.1
	Iceberg lettuce	41	96.0	0.002	0.080	0.024	0.020	9.8
	Chinese cabbage	21	96.2	0.004	0.042	0.015	0.012	0
Root/stem vegetables (excluding bulb vegetables)	Radish	293	94.0	ND	0.079	0.017	0.011	0
	Carrot	329	89.7	ND	0.116	0.021	0.018	0.6
	Kohlrabi	18	93.5	ND	0.016	0.007	0.005	0
	Potato	100	79.5	ND	0.088	0.026	0.019	0
	Ginger	12	94.3	ND	0.022	0.011	0.006	0
Gourd/fruit vegetables	Sponge gourd	100	95.2	ND	0.023	0.007	0.005	0
	Tomato	22	92.9	ND	0.044	0.008	0.014	0
	Cucumber	18	95.6	ND	0.115	0.010	0.011	0

[z] Moisture of iceberg lettuce and mustard adopt from recent data of TARI, while that of ceylon spinach source from USDA, http://www.nal.usda.gov/fnic/foodcomp/search/, and the remaining are from 'database of nutrient composition of food in Taiwan' published by Department of Health, Executive Yuan, R.O.C.

[y] SD: standard deviation.

0.294 mg kg^{-1}，(半) 結球菜類為ND–0.239 mg kg^{-1}，根莖菜類 (不含鱗莖類) 為ND–0.619 mg kg^{-1}，瓜菜及果菜類為ND–0.064 mg kg^{-1} (表3)。其中小葉菜類、(半) 結球菜類、瓜菜及果菜類之鉛濃度均在「蔬果植物類重金屬限量標準」之限值以內，根莖菜類 (不含鱗莖類) 的蘿蔔及胡蘿蔔分別有0.3%及4.0%樣本鉛濃度超出限值。以全部蔬菜數量計，鉛濃度超出限值者占0.7%。

不同蔬菜種類可食用部位鎘、鉛濃度有明顯差異。其中小葉菜類樣本鎘濃度平均值大小順序為菠菜 > 芹菜 > 紅鳳菜>茼蒿 > 皇宮菜 > 小白菜 > 油菜 > 青蔥 > 空心菜 > 青江菜 > 莧菜 > 芥菜 > 葉萵苣；鉛濃度平均值大小順序為菠菜 > 紅鳳菜 > 茼蒿 > 油菜 > 芹菜 > 青江菜 > 葉萵苣 > 空心菜 > 小白菜 > 青蔥 > 芥菜 > 皇宮菜 > 莧菜。(半) 結球菜類樣本鎘濃度平均值大小順序為 (半) 結球萵苣 > 高麗菜 > 大白菜；鉛濃度平均值大小順序為高麗菜 > 大白菜 > (半) 結球萵苣。根莖菜類 (不含鱗莖類) 樣本鎘濃度平均值大小順序為馬鈴薯 > 胡蘿蔔 >

表 3. 分析用蔬菜種類、樣品數、水分、鉛濃度 (包括最大、最小、平均及標準偏差值) 及超過國家標準值比率

Table 3. The species, sample size, water content, Pb concentration (values of maximum, minimum, mean and standard deviation), and rates of Pb concentration higher than the regulation levels in Taiwan

Vegetable group	Vegetable name	*n*	Moisture [z] (%)	Min.	Max.	Mean	SD [y]	Above the national
				(mg kg^{-1} fresh weight)				
Leaf vegetables	Pai-tsai	121	95.7	ND	0.096	0.031	0.026	0
	Spinach	18	93.0	0.051	0.170	0.111	0.038	0
	Mustard	86	94.6	ND	0.179	0.028	0.035	0
	Leafy lettuce	77	96.9	ND	0.196	0.045	0.048	0
	Water spinach	124	92.8	ND	0.197	0.037	0.033	0
	Amaranth	25	93.9	ND	0.044	0.014	0.012	0
	Pak-choi	101	94.8	ND	0.174	0.046	0.035	0
	Rape	24	95.4	ND	0.128	0.056	0.036	0
	Velvet plant	31	92.6	ND	0.294	0.081	0.066	0
	Ceylon spinach	16	93.1	ND	0.069	0.027	0.022	0
	Garlandchry santhemum	20	95.0	ND	0.192	0.065	0.051	0
	Celery	26	94.7	ND	0.161	0.051	0.044	0
	Green onion	228	92.2	ND	0.174	0.029	0.025	0
Head vegetables	Cabbage	49	93.5	ND	0.239	0.045	0.058	0
	Iceberg lettuce	41	96.0	ND	0.041	0.012	0.011	0
	Chinese cabbage	21	96.2	ND	0.091	0.028	0.020	0
Root/stem vegetables (excluding bulb vegetables)	Radish	293	94.0	ND	0.319	0.075	0.054	0.3
	Carrot	329	89.7	ND	0.619	0.114	0.089	4.0
	Kohlrabi	18	93.5	ND	0.053	0.017	0.020	0
	Potato	100	79.5	ND	0.150	0.038	0.023	0
	Ginger	12	94.3	ND	0.197	0.132	0.043	0
Gourd/fruit vegetables	Sponge gourd	100	95.2	ND	0.056	0.028	0.011	0
	Tomato	22	92.9	ND	0.064	0.014	0.017	0
	Cucumber	18	95.6	ND	0.055	0.020	0.015	0

[z] Moisture of iceberg lettuce and mustard adopt from recent data of TARI, while that of ceylon spinach source from USDA, http://www.nal.usda.gov/fnic/foodcomp/search/, and the remaining are from 'database of nutrient composition of food in Taiwan' published by Department of Health, Executive Yuan, R.O.C.

[y] SD: standard deviation.

蘿蔔 > 薑 > 結頭菜；鉛濃度平均值大小順序為薑 > 胡蘿蔔 > 蘿蔔 > 馬鈴薯 > 結頭菜。瓜菜及果菜類樣本鎘濃度平均值大小順序為花胡瓜 > 番茄 > 絲瓜；鉛濃度平均值大小順序為絲瓜 > 花胡瓜 > 番茄。

討　　論

與國內調查資料比較

Shih *et al*. (2008) 調查台北地區生鮮及傳統市場蔬菜可食用部位重金屬濃度，其鎘濃度範圍分別為高麗菜ND–0.003 mg kg^{-1}、芥菜0.004–0.010 mg kg^{-1}、小白菜ND–0.023 mg kg^{-1}、青江菜0.003–0.020 mg kg^{-1}、萵苣ND–0.021 mg kg^{-1}、馬鈴薯0.021–0.038 mg kg^{-1}，鉛濃度範圍分別為高麗菜ND、芥菜0.001–0.009 mg kg^{-1}、小白菜ND–0.036 mg kg^{-1}、青江菜ND–0.022 mg kg^{-1}、萵苣ND–0.012 mg kg^{-1}、馬鈴薯ND–0.004 mg kg^{-1}。本研究調查上述蔬菜之鎘濃度範圍則分別為高麗菜ND–0.061 mg kg^{-1}、芥菜ND–0.053 mg kg^{-1}、小白菜ND–0.115 mg kg^{-1}、青江菜ND–0.096 mg kg^{-1}、

萵苣ND–0.073 mg kg^{-1}、馬鈴薯ND–0.088 mg kg^{-1}，鉛濃度範圍分別為高麗菜ND–0.239 mg kg^{-1}、芥菜ND–0.179 mg kg^{-1}、小白菜ND–0.096 mg kg^{-1}、青江菜ND–0.174 mg kg^{-1}、萵苣ND–0.196 mg kg^{-1}、馬鈴薯ND–0.150 mg kg^{-1}，濃度分布範圍均較Shih *et al*. (2008) 數據為寬。若由調查樣本數量及採樣區域觀之，本研究所調查上述蔬菜樣本數量共534個，樣區分布全省各主要蔬菜產區，而Shih *et al*. (2008) 之樣本數量僅55個，採樣區域僅限台北地區，本研究之樣本應較有代表性。

Lin *et al*. (1992) 調查全省蔬菜專業區之蔬菜共346個樣本，其鎘濃度範圍分別為葉菜類ND–0.12 mg kg^{-1}、根菜類ND–0.91 mg kg^{-1}、果菜類ND–0.07 mg kg^{-1}，鉛濃度範圍分別為葉菜類ND–1.04 mg kg^{-1}、根菜類ND–1.00 mg kg^{-1}、果菜類ND–1.93 mg kg^{-1}。本研究調查之葉菜類[含小葉菜及 (半) 結球菜類]、根菜類 (含蘿蔔及胡蘿蔔)、果菜類樣本共1770個樣本，鎘濃度範圍則分別為葉菜類ND–0.151 mg kg^{-1}、根菜類ND–0.116 mg kg^{-1}、果菜類ND–0.115 mg kg^{-1}，鉛濃度範圍分別為葉菜類ND–0.294 mg kg^{-1}、根菜類ND–0.619 mg kg^{-1}、果菜類ND–0.064 mg kg^{-1}。除葉菜類及果菜類之鎘外，濃度分布範圍均較Lin *et al*. (1992) 數據為小。由Lin *et al*. (1992) 之數據亦可佐證，當樣本數量及採樣區擴大時，蔬菜鎘、鉛濃度分布範圍也會擴大，Shih *et al*. (2008) 之資料應較難代表整個台灣地區蔬菜之鎘、鉛濃度背景資料。

與國外調查資料比較

本研究與國外蔬菜鎘、鉛濃度比較詳如表4、表5。整體而言，本研究之蔬菜鎘、鉛濃度均高於Tahvonen & Kumpulainen (1995) 及Urieta *et al*. (1996) 之調查資料，前者之調查對象為芬蘭批發市場及食品廠，後者則以西班牙巴斯克自治區國人飲食研究樣本為對象，總樣本數分別為52及19。該兩項調查主要目的在瞭解人民由飲食中攝入重金屬的風險，其樣本來源為市場、食品加工廠，且部分樣品經過混樣、烹煮或調製，與本研究採自田間略有不同，且蔬菜樣本數較少，故難以做對等的比較。

Wolnik *et al*. (1985) 針對美國主要作物產區的蔬菜鎘、鉛濃度調查，Wiersma *et al*. (1986) 針對荷蘭主要作物產區的蔬菜鎘、鉛濃度調查，則與本研究之大面積區域性調查之屬性較為相近。由美國及荷蘭蔬菜鎘濃度中數值與本研究之比較，台灣地區蔬菜之鎘濃度與美國及荷蘭相近，若比較三個研究之各種蔬菜鎘濃度最大值，則除了荷蘭之高麗菜低於本研究外，本研究其他蔬菜鎘濃度最大值均小於上述兩研究之數值，顯示國內蔬菜鎘濃度與美國及荷蘭無明顯差異。另外，本研究蔬菜鉛濃度中數值與上述兩研究相較，除了荷蘭的 (半) 結球萵苣高於本研究外，本研究其他蔬菜鉛濃度中數值均高於上述兩研究之數值。由於廢棄石油與固體垃圾的焚化、煉鋼廠與煉鐵場、鉛的煉製、電池與烷基鉛的製造及民國89年無鉛汽油停用前交通工具排放出的廢氣，為台灣鉛污染主要污染源。鉛可藉大氣、落塵等方式散布，並經由植物葉面吸收 (Uzu *et al*. 2010)。台灣地狹人稠，人口密度約為荷蘭的1.5倍、美國的20倍，推測應為作物接觸藉由大氣或落塵飄散之鉛污染源的機會較高，導致台灣地區蔬菜鉛濃度高於美國及荷蘭。

Kachenko & Singh (2006) 調查澳洲新南威爾斯住商混合地區蔬菜產區蔬菜鎘、鉛濃度資料則顯示，除了高麗菜的鉛濃度最大值外，其餘蔬菜之鎘、鉛濃度最大值均明顯高於本研究。由於其四大採樣區中有兩個樣區鄰近金屬冶鍊廠，一個鄰近雪梨都會區，因此蔬菜中鎘、鉛濃度普遍偏高。另，由該研究不同採樣地點之蔬菜鎘、鉛濃度可發現，離工業及都會區較遠 (300 km) 的Cowra地區，其耕作環境較無人類活動之破壞，土壤中鎘、鉛濃度均極

低，蔬菜鎘濃度均符合澳洲食品限量標準，然而鉛濃度則仍有37.5%超出食品限量標準，顯示蔬菜作物鉛濃度與土壤濃度並無明顯相關，亦部分呼應鉛可藉大氣、落塵等方式散布，並經由植物葉面吸收之理論。

不同蔬菜種類鎘、鉛濃度比較

由蔬菜鎘、鉛濃度平均值顯示：不同類別蔬菜之平均鎘濃度依序為小葉菜類 > 根莖

表 4. 不同試驗蔬菜可食用部位鎘濃度比較

Table 4. Comparison of cadmium concentrations in edible parts of vegetables reported in various studies

		Concentrations (mg kg^{-1} fresh weight)					
Vegetable name	*n*	Min.	Max.	Mean	Median	Country	Literature
Chinese mustard	121	ND	0.115	0.023	0.018	Taiwan	Present study
	15	ND	0.023	—[z]	0.007	Taiwan	Shih *et al*. 2008
Spinach	18	0.024	0.133	0.074	0.079	Taiwan	Present study
	44	0.005	0.743	—	—	Australia	Kachenko & Singh 2006
	82	0.010	0.150	0.060	0.060	Netherland	Wiersma *et al*.1986
	104	0.012	0.195	0.065	0.0605	USA	Wolnik *et al*. 1985
Leafy lettuce	77	ND	0.073	0.019	0.014	Taiwan	Present study
	5	ND	0.021	—	0.008	Taiwan	Shih *et al*. 2008
Pak-choi	101	ND	0.096	0.022	0.018	Taiwan	Present study
	10	0.003	0.020	—	0.014	Taiwan	Shih *et al*. 2008
Cabbage	49	ND	0.061	0.016	0.009	Taiwan	Present study
	10	ND	0.003	—	ND	Taiwan	Shi *et al*. 2008
	8	0.003	0.062	—	—	Australia	Kachenko & Singh 2006
	8	0.001	0.007	0.003	—	Finland	Tahvonen & Kumpulainen 1995
	86	0.001	0.017	0.005	0.004	Netherland	Wiersma *et al*.1986
Iceberg lettuce	41	0.002	0.080	0.024	0.023	Taiwan	Present study
	56	0.005	0.424	—	—	Australia	Kachenko & Singh 2006
	13	0.006	0.029	0.012	—	Finland	Tahvonen & Kumpulainen 1995
	75	0.01	0.19	0.05	0.04	Netherland	Wiersma *et al*.1986
Chinese cabbage	21	0.004	0.042	0.015	0.011	Taiwan	Present study
	8	0.003	0.021	0.011	—	Finland	Tahvonen & Kumpulainen 1995
Carrot	329	ND	0.116	0.021	0.017	Taiwan	Present study
	12	0.002	0.074	0.024	—	Finland	Tahvonen & Kumpulainen 1995
	100	0.005	0.160	0.040	0.030	Netherland	Wiersma *et al*.1986
	207	0.002	0.130	0.028	0.017	USA	Wolnik *et al*. 1985
Potato	100	ND	0.088	0.026	0.025	Taiwan	Present study
	5	0.021	0.038	—	0.026	Taiwan	Shi *et al*. 2008
	19	0.005	0.061	—	—	Spain	Urieta *et al*. 1996
	11	0.001	0.016	0.006	—	Finland	Tahvonen & Kumpulainen 1995
	94	0.002	0.090	0.030	0.030	Netherland	Wiersma *et al*.1986
Tomato	22	ND	0.044	0.008	0.001	Taiwan	Present study
	9	ND	0.004	0.001	—	Finland	Tahvonen & Kumpulainen 1995
Cucumber	18	ND	0.115	0.010	0.004	Taiwan	Present study
	10	ND	ND	ND	—	Finland	Tahvonen & Kumpulainen 1995

[z] '—' denotes 'no data.'

菜類 (不含鱗莖類) > (半) 結球菜類 > 瓜菜及果菜類；平均鉛濃度依序為根莖菜類 (不含鱗莖類) > 小葉菜類 > (半) 結球菜類 > 瓜菜及果菜類。與前人研究結果相較 (Howe *et al*. 2005; Lin *et al*. 1992; Murray *et al*. 2011; Shih *et al*. 2008; Yang *et al*. 2009)，蔬菜類別間鎘濃度排序互有高低，應與各研究所分析蔬菜種類不完全相同有關，然整體而言，多數結果均顯示果

表 5. 不同試驗間蔬菜可食用部位鉛濃度比較

Table 5. Comparison of lead concentrations in edible parts of vegetables reported in various studies

Vegetable name	*n*	Concentrations (mg kg^{-1} fresh weight)				Country	Literature
		Min.	Max.	Mean	Median		
Chinese mustard	121	ND	0.096	0.031	0.024	Taiwan	Present study
	15	ND	0.036	— [z]	0.004	Taiwan	Shih *et al*. 2008
Spinach	18	0.051	0.325	0.120	0.120	Taiwan	Present study
	44	ND	11.6	—	—	Australia	Kachenko & Singh 2006
	82	0.010	0.029	0.090	0.080	Netherland	Wiersma *et al*.1986
	104	0.016	0.170	0.045	0.039	USA	Wolnik *et al*. 1985
Leafy lettuce	77	ND	0.357	0.045	0.039	Taiwan	Present study
	5	ND	0.012	—	0.002	Taiwan	Shih *et al*. 2008
Pak-choi	101	ND	0.174	0.046	0.039	Taiwan	Present study
	10	ND	0.022	—	0.002	Taiwan	Shih *et al*. 2008
Cabbage	49	0.003	0.239	0.045	0.023	Taiwan	Present study
	10	ND	ND	—	ND	Taiwan	Shih *et al*. 2008
	8	ND	0.222	—	—	Australia	Kachenko & Singh 2006
	8	ND	0.002	0.001	—	Finland	Tahvonen & Kumpulainen 1995
	86	0.002	0.230	0.015	0.006	Netherland	Wiersma *et al*.1986
Iceberg lettuce	41	ND	0.041	0.012	0.011	Taiwan	Present study
	56	ND	4.87	—	—	Australia	Kachenko & Singh 2006
	13	0.002	0.007	0.004	—	Finland	Tahvonen & Kumpulainen 1995
	75	0.03	2.25	0.140	0.090	Netherland	Wiersma *et al*.1986
Chinese cabbage	21	0.006	0.091	0.028	0.023	Taiwan	Present study
	8	ND	0.004	0.001	—	Finland	Tahvonen & Kumpulainen 1995
Carrot	329	ND	0.619	0.114	0.100	Taiwan	Present study
	12	0.001	0.012	0.006	—	Finland	Tahvonen & Kumpulainen 1995
	100	0.011	0.210	0.050	0.040	Netherland	Wiersma *et al*.1986
	207	0.001	0.125	0.009	0.0065	USA	Wolnik *et al*. 1985
Potato	100	ND	0.150	0.038	0.035	Taiwan	Present study
	5	ND	0.004	—	ND	Taiwan	Shih *et al*. 2008
	19	0.0025	0.035	—	—	Spain	Urieta *et al*. 1996
	11	0.004	0.007	0.005	—	Finland	Tahvonen & Kumpulainen 1995
	94	0.01	0.08	0.030	0.030	Netherland	Wiersma *et al*.1986
Tomato	22	ND	0.064	0.014	0.010	Taiwan	Present study
	9	0.001	0.002	0.0015	—	Finland	Tahvonen & Kumpulainen 1995
Cucumber	18	ND	0.055	0.020	0.017	Taiwan	Present study
	10	ND	0.001	ND	—	Finland	Tahvonen & Kumpulainen 1995

[z] '—' denotes 'no data.'

菜類及豆菜類作物之鎘濃度較低，葉菜類濃度較高；類別間鉛濃度排序則變動較大，不同研究間難以歸納出共同的趨勢，推測應與鉛可經由植物葉面吸收，易受大氣中飄散的懸浮污染源影響有關。

過去的研究顯示，蔬菜作物對鎘吸收能力有明顯差別 (Alloway *et al*. 1988; Kachenko & Singh 2006; Nabulo *et al*. 2006; Tahvonen & Kumpulainen 1995; Yang *et al*. 2009)。整體而言，菠菜、芹菜屬於吸收鎘能力較強的作物，(半) 結球萵苣、韭蔥、胡蘿蔔對鎘具有中等吸收能力，馬鈴薯、高麗菜、番茄、胡瓜則屬於吸收鎘能力較低的蔬菜。本研究之結果亦顯示，同一類別蔬菜中不同蔬菜種類之鎘濃度有很大差異 (圖2)。以小葉菜類為例，其中菠菜鎘濃度明顯高於其他蔬菜，與前人研究菠菜屬於鎘吸收能力較強之蔬菜結果相符；本研究芹菜樣本鎘濃度僅略高於其他蔬菜，與國外研究結果頗有差距，推測應與台灣主要栽培之芹菜以葉芹為主，而非歐美所栽培葉柄肉厚多汁的西洋芹，故對鎘吸收能力不相同。Yang *et al*. (2010) 研究20種蔬菜作物鎘吸收，其中芹菜的鎘吸收能力也僅屬中等。另外，本研究之 (半) 結球萵苣及高麗菜樣本分別有9.8%及6.1%超過我國「蔬果植物類重金屬限量標準」(Department of Health, Executive Yuan 2011) 0.05 mg kg^{-1}，與前人研究 (半) 結球萵苣、高麗菜對鎘具有中、低等吸收能力頗有出入。分析其採樣位置顯示，(半) 結球萵苣來自11個樣區，超過限值的4個樣本均來自同一樣區，由過去土壤調查資料顯示 (未發表)，該樣區土壤以0.01 M氯化鈣萃取鎘濃度 (0.025 mg kg^{-1} ± 0.003 mg kg^{-1}) 確高於其他樣區 (0.009 mg kg^{-1} ± 0.005 mg kg^{-1})；高麗菜來自11個樣區，超過限值的5個樣本亦來自同一樣區，該樣區土壤鎘濃度 (0.066 mg kg^{-1} ± 0.016 mg kg^{-1}) 亦高於其他樣區 (0.010 mg kg^{-1} ± 0.005 mg kg^{-1})。由前人研究亦顯示，作物鎘濃度與土壤鎘濃度呈正相關 (Brennan & Mann 2005; Chen *et al*. 2010; Yang *et al*. 2009)。由此推測，本研究 (半) 結球萵苣及高麗菜之鎘濃度偏高，應受土壤鎘濃度之影響。

根據過去研究顯示，鉛被作物吸收後，大部分將存在根部 (Gopal & Rizvi 2008; Liu *et al*. 2010; Sahi *et al*. 2002)，且其可經由葉面吸收 (Uzu *et al*. 2010)，植體濃度常受含鉛落塵飄散的影響 (Nabulo *et al*. 2006)，因此，蔬菜食用部位鉛累積能力雖隨品種品系而有所差異 (Alexander *et al*. 2006; Gzyl 1990; Wang *et al*. 2006)，然變異性較大。本研究結果亦顯示，同一類別蔬菜中不同蔬菜種類之鉛濃度有很大差異 (圖2)，除其中蘿蔔與胡蘿蔔樣本鉛濃度分別有0.3%及4.0%超過我國「蔬果植物類重金屬限量標準」0.3 mg kg^{-1}外，其他蔬菜樣本鉛濃度均在我國限量標準以內。蘿蔔與胡蘿蔔樣本鉛濃度較高，推測應與鉛較易累積於作物根部有關。

結　　論

由本研究之普查資料顯示，大多數蔬菜鎘、鉛濃度均符合衛生署公告的「蔬果植物類重金屬限量標準」。然而，蔬菜對於鎘、鉛吸收能力不同，即使同一類別之不同種蔬菜其鎘、鉛濃度亦存在不小的差異，故消費者選購蔬菜種類應多樣化，可無鎘、鉛食用過量之疑慮。其次，本研究蔬菜樣本採自台灣本島主要蔬菜產區，採樣點均非環保署所公告之污染控制場址，理論上應無重金屬安全之虞，卻仍有部分蔬菜樣本鎘、鉛濃度超出「蔬果植物類重金屬限量標準」。其中鎘吸收與土壤鎘濃度成正相關，鉛吸收則與土壤鉛濃度無明顯相關，顯示後續研究有必要深入探討台灣地區不同品種蔬菜鎘、鉛吸收、傳輸、分配機制，以及土壤理化性質、環境因子對鎘、鉛吸收之影響機制，以作為相關土壤污染及食品安全法規研擬或修正之參考。

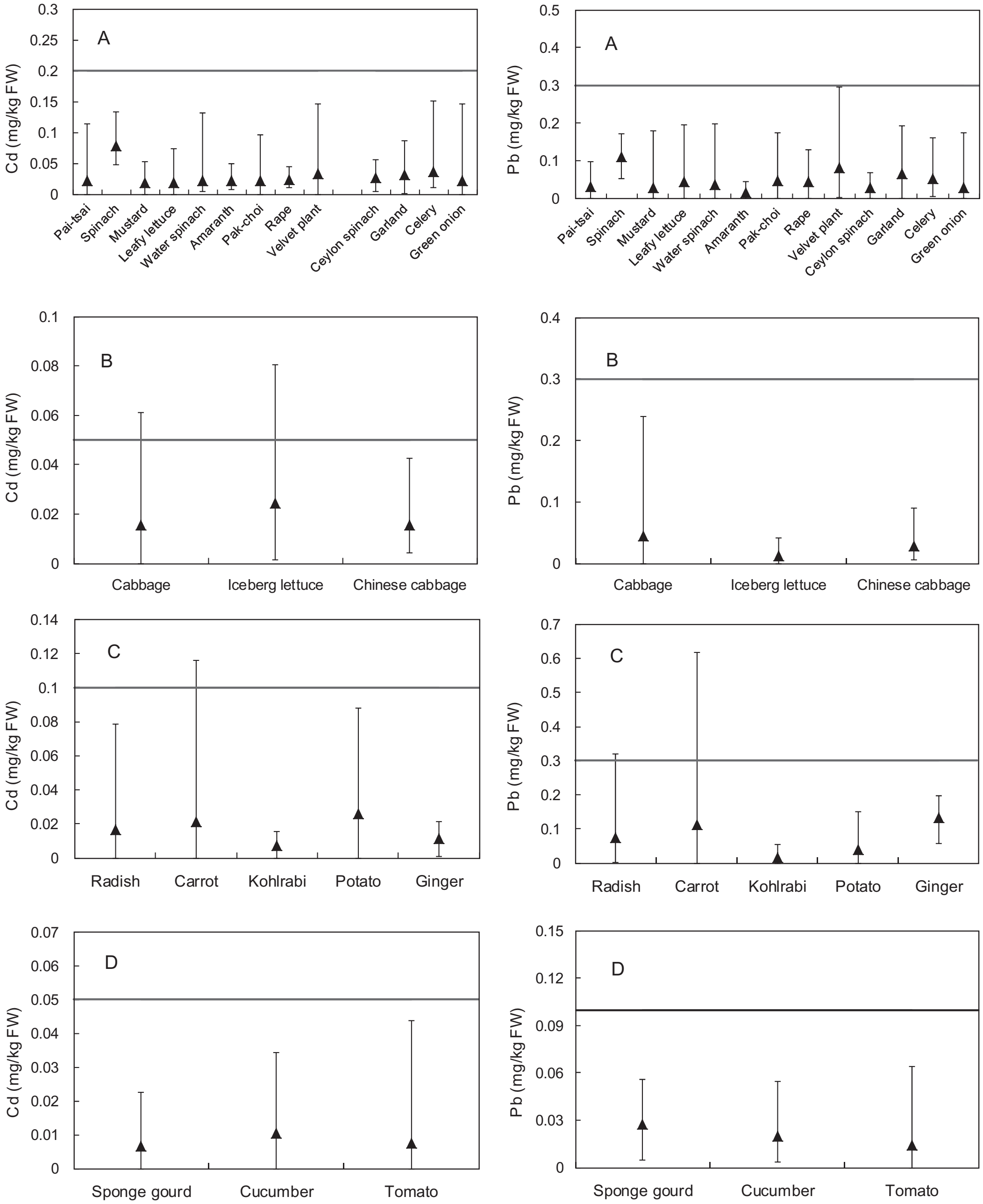

圖 2. 各類別蔬菜鎘、鉛濃度圖 [(A) 葉菜類，(B) (半) 結球菜類，(C) 根莖菜類，(D) 瓜菜及果菜類；各蔬菜濃度範圍以上下誤差線表示；水平實線為台灣蔬果植物類鎘、鉛限量標準]。

Fig. 2. Cadmium (left column, A–D) and lead (right column, A–D) concentrations in different groups of vegetable. (A) leaf vegetables; (B) head vegetables; (C) root/stem vegetables; (D) gourd/fruit vegetables. In each Figure, the concentration range of each crop is indicated by an error bar; the horizontal solid line denotes the food regulation levels for cadmium and lead in vegetable crops in Taiwan.

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Contents of Cadmium and Lead in Vegetable Crops Produced in Taiwan[1]

Yu-Wen Lin[2], Tsang-Shen Liu[2,10], Chi-Tsun Chen[3], Hsueh-Jung Tang[4], Hong-Tang Chen[5], Jia-Rong Job[6], Jeng-Hsien Tsai[7], Yong-Hong Lin[8], Chi-Chung Chang[9], Shwu-Jene Tsai[2], and Wei-Tin Huang[2]

Abstract

Lin, Y. W., T. S. Liu, C. T. Chen, H. J. Tang, H. T. Chen, J. R. Job, J. H. Tsai, Y. H. Lin, C. C. Chang, S. J. Tsai, and W. T. Huang. 2012. Contents of cadmium and lead in vegetable crops produced in Taiwan. J. Taiwan Agric. Res. 61:38–51.

In order to understand contents of cadmium and lead in vegetable crops produced in Taiwan, a total of 1900 samples of vegetable crops were collected from major vegetable growing areas in Taiwan and used in this study. These samples were digested by concentrated nitric acid and perchloric acid and then analyzed for contents of cadmium and lead, using inductively coupled plasma atomic emission spectrometer. Results showed that mean concentrations of cadmium (fresh weight basis) were ND–0.151 mg kg^{-1} in leaf vegetables, ND–0.080 mg kg^{-1} in head vegetables, ND–0.116 mg kg^{-1} in root/stem vegetables, and ND–0.115 mg kg^{-1} in gourd/fruit vegetables, whereas mean concentrations of lead were ND–0.294 mg kg^{-1} in leaf vegetables, ND–0.239 mg kg^{-1} in head vegetables, ND–0.619 mg kg^{-1} in root/stem vegetables, and ND–0.064 mg kg^{-1} in gourd/fruit vegetables. Results also showed that 0.6%

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and 0.7% of vegetable samples exceeded the food regulation levels for cadmium and lead in Taiwan, respectively. We particularly emphasized that 6.1%, 9.8%, and 0.6% of cabbage, iceberg lettuce, and carrot samples exceeded the regulation levels for cadmium, respectively, while 0.3% and 4.0% of radish and carrot samples exceeded those for lead, respectively.

Key words: Cadmium, Lead, Vegetable crops, Food safety.

台灣農業研究 (J. Taiwan Agric. Res.) 61(1):52–63 (2012)

芥藍與油菜種間雜種的獲得與鑑定 [1]

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摘　　要

邱金春、林子凱、林楨祐、夏奇鈮、王三太。2012。芥藍與油菜種間雜種的獲得與鑑定。台灣農業研究 61:52–63。

遠緣雜交可以擴大物種間基因的轉移，獲得種內雜交難以得到的基因型而進行作物的品種改良。芥藍 (*Brassica oleracea* var. *aboglabra*) 與油菜 (*Brassica campestris* ssp. *chinensis*) 經由蕾期人工授粉，同時進行正交與反交，摘取敗育前的未成熟胚，於經修正之MS培養基分別培育出種間雜交種。*B. oleracea*與*B. campestris*雜種胚培養成功率受胚齡、培養基組成、親本基因型的影響，同一組育種親本其正反與反交胚培養成功率及生育反應不同，以*B. campestris*為母本之雜交組合胚拯救成功率較高，說明母本基因型不同對雜種的誘導率有一定的影響。雜交實生苗經流式細胞儀檢定核酸含量確定為雜交後代，其外表性狀介於兩親之間但有偏雌效應的現象。

關鍵詞：芥藍、油菜、種間雜種、胚拯救。

前　　言

蕓苔屬蔬菜育種除了在亞種間雜交進行品種改良外，亦可經由遠緣雜交由他種導入遺傳質以獲得種內雜交難以得到的新基因類型。自從1980年以二倍體*Brassica oleracea*與*Brassica rapa*人工雜交形成異源四倍體*Brassica napus*葉菜類‘Hakuran’後，陸續以胚培養及子房培養育出多個十字花科種間 (interspecific) 雜種及屬間 (intergeneric) 雜種 (Kudou *et al*. 1995; Sarmah & Sarla 1998; Wang *et al*. 2008a, 2008b; Wen *et al*. 2008a, 2008b; Zhang *et al*. 2004)，藉由遠緣雜交技術不僅開發新種質，而且可將抗蟲性、早熟性、抗逆特性等重要經濟性狀轉移熟性至目標作物，例如種間雜交種*B. napus*分別自親本*Brassica oleracea*導入自交不親合性與抗甘藍蚜蟲 (Quazi 1988)；甘藍自*Brassica juncea*導入抗黑腐病特性 (Tonguc & Griffiths 2004)；白菜‘Komatsuna’與甘藍的雜交種再回交親本‘Komatsuna’，選育出抗Fusarium的後裔‘YR-Edogawa’ (Noguchi 2004)；*B. oleracea*由*Brassica carinata*導入抗白粉病特性；由*B. napus*轉移抗殺草劑三氮六環衍生物 (triazine) 至*B. oleracea*，轉移抗白銹病Race-7至*B. rapa*

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(Ayotte *et al*. 1987)；經由黃籽二倍體*B. rapa*與*B. oleracea*雜交獲得黃籽*B. napus* (Wen *et al*. 2008a)；經由種間雜交將*B. napus*的不產生芥酸的對偶基因導入*B. oleracea* (Bennett *et al*. 2008)；在雄不稔系育種中，遠緣雜交更是蔬菜獲得雄不稔特性的重要途徑，結球白菜 (*Brassica campestris* spp. *pekinensis* Olsson) 細胞質雄不稔 (cytoplasmic male sterility, CMS) 基因可轉至羽衣甘藍 (*B. oleracea* var. *acephala* DC.) (Zhu & Wei 2009)，蘿蔔CMS基因可導入蕓苔屬作物 (Paulmann & Röbbelen 1988; Sakai & Imanura 1990) 等。

蕓苔屬植物有A、B和C不同基因組，經由分子層次鑑定分類，已知蕓苔屬不同基因組中A和C親緣關係較A和B或B和C更為接近 (Inomata 2007; Yang *et al*. 2008)。而種原的親疏遠近對於遠緣雜交親本間的親和性具有決定性的影響 (Inomata 2007; Wang *et al*. 2008a)，遠緣雜交的兩親本因染色體同源性低，因此雜交過程中會出現各種障礙，包括有雜交不親和、胚敗育和雜種不稔性等。現在已知發育初期的未成熟胚培養是克服胚敗育最佳途徑之一，可以避免雜交胚的萎縮退化。影響胚培養成功與否的因素很多，尤其是種間雜交的親和性及胚珠退化時間會隨著雜交組合親本的不同而有差異，親本的基因型對其有很大的影響，在雜種胚敗育發生之前，掌握適當的受精胚摘取時間是影響胚培養成功與否的關鍵因子之一 (Inomata 2007; Yang *et al*. 2008)。此外，培植體對培養基組成的要求也會因為不同基因型親本和雜交組合，與培植體切離母體時胚的發育程度而有差異性。本試驗主要探討不同胚齡的雜種胚對*B. oleracea*與*B. campestris*種間雜交種培育的影響及胚培養的最佳生長條件。

材料與方法

試驗材料

供試的材料來自於農試所作物組與鳳山分所收集之蔬菜種原，包括芥藍 (*B. oleracea* var. *aboglabra*) 品系‘1506’、‘1507’、‘1508’、西農芥藍 (CN) 與油菜 (*B. campestris* ssp. *chinensis*) ‘B-01’、‘B-02’、‘B-03’、‘B-04’等品系。種子經播種萌芽後種植於栽培盆，定期施肥管理，植株於溫室內培育至開花。以不同品系芥藍與油菜進行正交與反交，其中西農芥藍具細胞質雄不稔性只作為育種母本。

雜交授粉

父本選擇開花前一天之花蕾先進行套袋避免污染，母本則於授粉當天早上選擇約一至兩天後開花之花蕾，除去花瓣及雄蕊，然後取開花當天採集之父本新鮮花粉沾在母本供試植株之柱頭上，套袋隔離，於授粉後7–20日分別取果莢觀察胚發育情形，並培育不同胚齡的呈現褐色果莢供胚培養。

胚培養

採用未成熟胚培養進行雜種胚拯救，分別摘取授粉後第10日、15日的幼嫩果莢，用75%之酒精浸漬數秒後經蒸餾水洗滌，再以0.5%次氯酸鈉溶液於超音波震盪10分鐘，經無菌水洗滌3次後取出材料，以滅菌後的解剖刀切開果莢，放入含NAA (0、0.1、1 ppm)、Kinetin (0、0.1 ppm)、casein hydroylsate 400 ppm及蔗糖5%之MS基本培養基，於22℃ ± 1℃培養室中以弱光進行培養並調查可發育胚數；待胚膨脹萌芽後，移入含$AgNO_3$ 0.5 ppm、Kinetin 0.5 ppm、NAA 0.1 ppm、casein hydroylsate 1000 ppm及蔗糖3%之MS培養基，置於2000 lux光照下繼續培育成苗，然後切取莖頂以側芽增殖方式將每個雜種植株繁殖成單株試管營養系。

種間雜種鑑別

待試管苗根長約0.5–1 cm時進行兩週的馴化，再移出管外以培養土栽培種植於溫室中。待植株達15 cm高時，取新芽之第一片葉展開後切取約1 cm^2葉片，加入0.2 mL UV CyStain Precise T萃取緩衝液A (Kit from Partec, Ger-

many)，以刀片將組織切碎過濾，再於濾液中添加0.8 mL Partec Cystain Precise T緩衝液B，靜置染色3分鐘。每樣品取樣2次，以流式細胞儀 (flow cytometry, Partec PA, Germany) 分析系統，檢測5000個細胞核，分析細胞內之核酸含量。

結 果

雜交胚的發育與培養適期

以‘1506’等4個不同芥藍品系與‘B-01’等4個不同油菜品系進行*B. oleracea*與*B. campestris*種間雜交蕾期授粉，無論是正交或反交，授粉後初期子房明顯膨大並轉綠，但隨著授粉後日數增加，子房發育呈現停滯然後逐漸黃化與脫落，最終都無法於活體植株 (*in vivo*) 獲得雜交種子。探討授粉後不同時期胚的發育，可以幫助決定適當胚摘取時間。母本花朵於授粉後5–20日定期切開幼嫩果莢可觀察到雜交胚的發育過程，在授粉後第5–7日可由幼嫩之果莢內部觀察到呈半透明之淡綠色多細胞的球型胚；在授粉後第二週球型胚數量會略增加，胚也漸轉為綠色；但是在授粉後第14日起多數雜交胚在外型上呈扁平化，色澤轉為淡褐色；在授粉後第20日已可觀察到多數雜交胚有皺縮現象，色澤更暗呈現褐色，此時果莢外觀也多為黃化甚至脫落 (圖1)。遠緣雜交育種常因親本間親緣性低，造成兩親本染色體配對困難，使得雜種胚在授粉受精後於發育過程中容易發生胚敗育，授粉後日數越多，敗育的雜種胚數目越多，平均每個幼嫩果莢能順利摘取進行培養的胚數量驟減，但是胚成熟度會影響胚培養成活率，因此掌握適當胚摘取時期非常重要。授粉後第1週雜交胚成熟度普遍不足，授粉第4週後的雜交胚多萎縮呈暗褐色，不僅培植體取得不易且已敗育的雜交胚也難再進行胚拯救；授粉後7–20日的蕓苔屬種間雜交胚都可能經培養而發育成植株，隨著日齡的增加胚發育越趨成

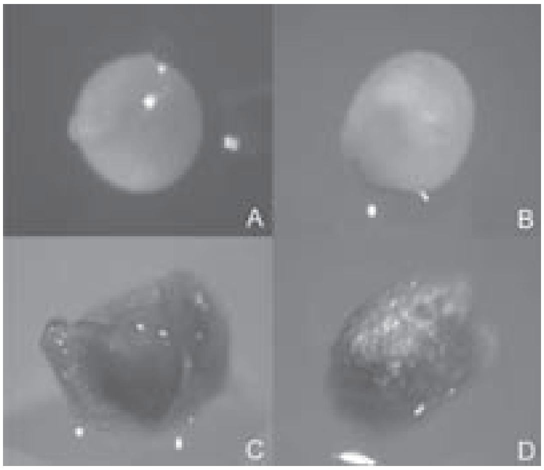

圖 1. 不同發育階段的油菜 × 芥藍種間雜交胚，(A–D)：雜交胚分別為授粉後 5 日、10 日、15 日及 20 日齡。

Fig. 1. Development of hybrid embryos from *Brassica campestris* ssp. *chinensis* × *B. oleracea* var. *aboglabra* after pollination 5 (A), 10 (B), 15 (C) and 20 (D) days.

熟培養成功率也越高，唯多數邁入第三週齡的遠緣雜交胚常急速轉褐萎縮，果莢內部能摘取培養的雜交胚數目驟減甚至胚完全萎縮死亡無法進行胚拯救。親本基因型會影響遠緣雜交胚衰敗速率，不同油菜品系為母本與同一芥藍品系雜交，‘B-02’ × ‘1507’子房發育期較‘B-04’ × ‘1507’長，‘B-03’ × ‘1508’子房發育期較‘B-01’ × ‘1508’為長，因此‘B-02’ × ‘1507’與‘B-03’ × ‘1508’可取得授粉後20日齡胚進行培養，但是‘B-04’ × ‘1507’與‘B-01’ × ‘1508’子房內部胚已完全萎縮敗育 (表1)。為兼顧培植體取得及胚拯救成功率，因此本試驗分別以授粉後10日、15日與20日齡子房摘取內部的未成熟胚作為胚培養材料來源，唯雜交胚培養適期因親本品系不同而有差異。

親本基因型、正反交及培養基組成對培育種間雜種之影響

以不同品系油菜作為母本與同一芥藍品系雜交，‘B-02’ × ‘1507’培植體生長反應較‘B-04’ × ‘1507’為佳，另‘B-03’ × ‘1508’培植體

表 1. 不同品系油菜母本在油菜與芥藍遠緣雜交胚培養之效果

Table 1. The effect of different parent lines on embryo culture of *Brassica campestris* ssp. *chinensis* × *B*. *oleracea* var. *aboglabra*

		B. campestris ssp. *chinensis* × *B. oleracea* var. *aboglabra*			
Embryo (DAF) [z]	Regulator [y]	‘B-02’ × ‘1507’	‘B-04’ × ‘1507’	‘B-01’ × ‘1508’	‘B-03’ × ‘1508’
10	K 0.1, N 1	0/6 [x]	1/7	1/10	0/29
10	K 0.1, N 0.1	0/6	0/7	3/10	2/28
10	K 0, N 1	0/6	0/7	1/10	0/28
10	K 0, N 0.1	1/6	0/7	0/10	1/29
10	K 0, N 0	0/7	0/8	1/10	0/29
15	K 0.1, N 1	1/4	0/2	0/2	3/9
15	K 0.1, N 0.1	2/3	0/2	0/2	2/9
15	K 0, N 1	1/4	0/2	0/2	2/9
15	K 0, N 0.1	2/5	0/2	1/2	3/9
15	K 0, N 0	3/5	0/2	1/2	2/9
20	K 0.1, N 1	2/2	—	—	0/5
20	K 0.1, N 0.1	1/2	—	—	0/5
20	K 0, N 1	1/2	—	—	0/5
20	K 0, N 0.1	1/2	—	—	0/5
20	K 0, N 0	2/2	—	—	0/5

[z] DAF means days after pollination.
[y] Concentration of kinetin (K) and NAA (N) in ppm.
[x] Number of plantlets/number of cultured embryos.

則較‘B-01’ × ‘1508’容易形成雜交單株 (表1)，胚培養結果顯示雜種胚培養成功率受母本不同基因型的影響。不同品系芥藍與油菜進行種間雜交授粉，獲得正交與反交共14個雜交組合，胚培養結果多數都有發育為雜交單株 (表2)，少數則形成癒合組織伴有根的分化；其中僅3組胚培養後培植體沒有任何生長反應，包括‘1508’ × ‘B-03’、‘1507’ × ‘B-02’是以芥藍作為母本，‘B-01’ × ‘1507’則是以油菜為母本之雜交組合。*B. oleracea*與*B. campestris*種間雜交正交與反交胚培養後培植體生育是不同的，即使同一組育種親本在相同基因型的前提下進行正反交，以油菜為母本的5個雜交組合胚培養成功率較以芥藍為母本的反交組合高，雜交胚於培養基多直接形成雜交單株。以芥藍為母本與不同品系油菜父本雜交，雜交胚培養除成功率較低外，6個有生長反應雜交組合中有5個組合培植體僅有根形成，也高於以油菜為母本的雜交組合 (表2)，而正反交的根形成並無特定組合較高比例關係。西農芥藍具細胞質雄不稔性只能作為育種母本，經授粉後胚培養結果僅‘CN’ × ‘B-01’形成種間雜交單株，另兩組培植體僅長根無地上部發生。此試驗中不同品系芥藍與油菜正反交結果，8個雜交組合中僅油菜‘B-01’ × 芥藍‘1508’ 1個組合雜交株具有花粉，經蕾期授粉自交，目前已產生第一代種子，其餘7個組合均為不稔的雜交株。

除了胚成熟度外，培養基中植物生長調節劑的施用也是遠緣雜交胚成活率的影響因子之一。在不含植物生長調節劑之基本培養基中，胚培養也可以育苗，但以日齡較多的雜交胚為宜；僅添加NAA (0.1–1 ppm) 對*B. campestris*與*B. oleracea*種間雜交胚拯救的效果並不明顯，但是同時添加NAA與Kinetin 0.1 ppm則可提高

表 2. 不同品系油菜與芥藍的種間雜交胚培養植株/根之再生率

Table 2. Rate of hybrids/roots regeneration through embryo culture of different lines from crosses between *Brassica campestris* and *Brassica oleracea*

Crosses	Plantlets formed (%)				
	K 0.1, N 1 [z]	K 0.1, N 0.1	K 0, N 1	K 0, N 0.1	K 0, N 0
B. oleracea var. *aboglabra* × *B. campestris* ssp. *chinensis*					
‘CN’ × ‘B-01’	20.0	0.0	0.0 (30.0)	0.0	0.0
‘CN ’× ‘B-02’	0.0 (35.7) [x]	0.0	0.0 (15.4)	0.0	0.0
‘CN’ × ‘B-03’	0.0 (6.7)	0.0 (6.7)	0.0	0.0	0.0
‘1507’ × ‘B-02’	0.0	0.0	0.0	0.0	0.0
‘1508’ × ‘B-03’	0.0	0.0	0.0	0.0	0.0
‘1506’ × ‘B-01’ [y]	0.0	0.0	0.0	0.0 (1.9)	1.9
‘1507’ × ‘B-04’ [y]	0.0 (5.3)	0.0	0.0 (5.3)	0.0	0.0
‘1508’ × ‘B-01’ [y]	0.0	0.0	0.0 (4.0)	0.0	4.0
B. campestris ssp. *chinensis* × *B. oleracea* var. *aboglabra*					
‘B-01’ × ‘1506’ [y]	28.6	7.1	15.4	15.4	7.7
‘B-04’ × ‘1507’ [y]	11.1	0.0 (22.2)	0.0	0.0	0.0
‘B-01’ × ‘1508’ [y]	8.3	25.0	8.3	8.3	16.7
‘B-01’ × ‘1507’	0.0	0.0	0.0	0.0	0.0
‘B-02’ × ‘1507’	25.0	27.3	16.7 (8.3)	30.8	35.7
‘B-03’ × ‘1508’	7.0 (4.7)	9.5 (2.4)	4.8 (2.4)	9.3	4.7

[z] Concentration of kinetin (K) and NAA (N) in ppm.
[y] Percentage of root formation from explants.
[x] Reciprocal hybridization.

雜交胚成活率，尤其對成熟度不足的10日齡胚拯救促進效果較15日齡與20日齡胚顯著，其中同時添加NAA 0.1 ppm與Kinetin 0.1 ppm胚培養育苗效果略優於NAA 1 ppm與Kinetin 0.1 ppm處理者 (表1)。不同品系*B. campestris*與*B. oleracea*種間雜交胚培養成功率有差異性，部分雜交組合培植體培養後形成癒合組織僅有根分化而無莖芽的生長。在植物生長調節劑輔助之下，以*B. campestris*為育種母本者胚拯救成功率為4.7–35.7%，都高於其反交組合，植物生長調節劑的添加對以*B. campestris*為母本地雜交胚拯救似乎沒有很明顯的影響，即使在不含植物生長調節劑的基本培養基中也能部分萌芽；但是對以芥藍為母本的雜交組合，於含有NAA 0.1–1 ppm之培養基中則明顯有形成癒合組織及根分化的趨勢，而抑制了雜交胚的萌發，此現象於NAA濃度達1 ppm時更明顯 (表2)。

種間雜種之鑑別

進一步以流式細胞儀分析試驗中各雜交組合的親本及其種間雜種的核酸含量，結果顯示所有種間雜種之相對DNA含量均介於雙親之間；圖2所示為油菜‘B-03’與芥藍‘1508’雜交組合中，親本與雜種後裔之核酸含量分析結果，若將母本油菜‘B-03’之DNA含量設為基準值，則油菜‘B-03’、‘B-03’ × ‘1508’雜種及芥藍‘1508’三者於細胞週期G1 stage測得之平均螢光讀值分別為100、131及154，而於G2 stage時則依序為200、253及303，該種間雜種的相對DNA含量介雙親之間且趨近於雙親之平均值 (圖2)，本試驗依流式細胞儀分析結果可清楚鑑別出種間雜交株。

雜交後裔之外表型

由胚培養獲得的*B. campestris*與*B. oleracea*種間雜交株，經馴化處 後已移出管外並種植於溫室，雜交株生長勢強。芥藍為*B. oleracea*的變種之一，葉片為暗或淺灰綠色，葉質較厚；油菜又稱小油菜、白菜型油菜，為*B. campestris*的變種之一，植株矮小，葉質柔軟質地較薄，葉形為近圓或橢圓形，葉淺綠色或黃綠色；雜交株葉色、葉片質地等性狀介於油菜與芥藍之間，以*B. oleracea*為授粉母本則雜交株的葉片略帶革質、葉色呈現暗綠；以*B. campestris*為母本則雜交株葉片質地較薄、葉色較淺綠(圖3)。

討　　論

蕓苔屬主要由3個不同基因組的基本種和3個複合種及衍生的變種組成，近年來經由分子層次的系譜分析得知，蕓苔亞族植物可以明顯地區分為*B. nigra*與*B. oleracea/B. campestris*兩大族群，表示不同基因組中A和C基因組之間的親緣關係較A和B或B和C更為接近 (Song *et al*. 1988; Warwick & Black 1993; Yang *et al*. 2008)。分屬於*B. oleracea*與*B. campestris*的芥藍與油菜親緣性雖然較A與B基因組或B與C基因組更接近，但在自然狀況下仍難以獲得雜交種子，因此有需要藉助胚拯救技術 (Huang *et al.* 2008; Kudou *et al*. 1995; Man *et al*. 2007;

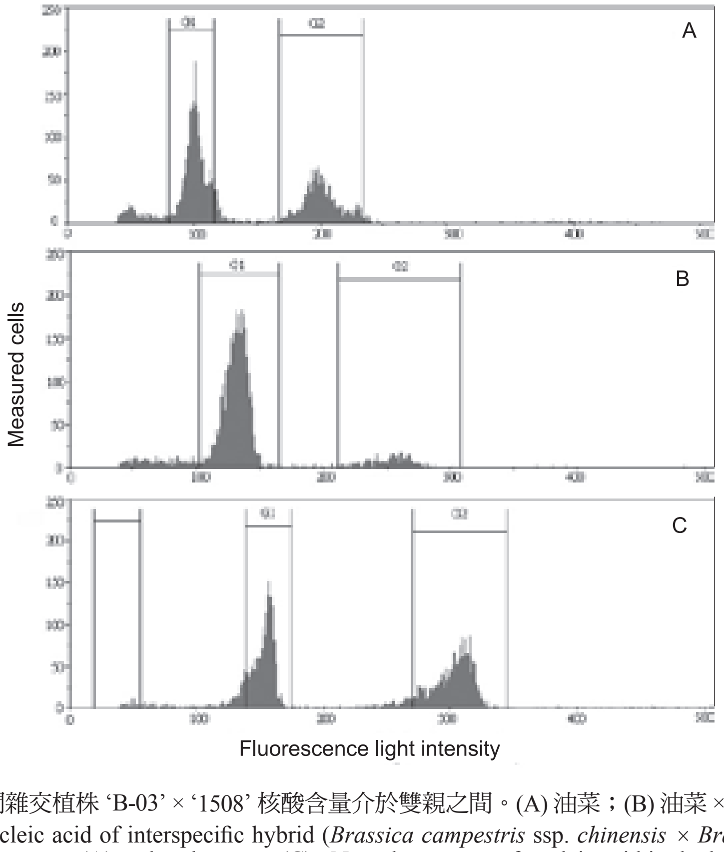

圖 2. 油菜與芥藍種間雜交植株 ‘B-03’ × ‘1508’ 核酸含量介於雙親之間。(A) 油菜；(B) 油菜 × 芥藍；(C) 芥藍。

Fig. 2. Amount of nucleic acid of interspecific hybrid (*Brassica campestris* ssp. *chinensis* × *Brassica oleracea* var. *aboglabra*) (B), female parent (A) and male parent (C). Note the amount of nucleic acid in the hybrid is between the parents.

圖 3. 油菜與芥藍種間雜交植株其外表型態介於兩親本之間。(A) 由左至右分別為油菜、雜交株及芥藍葉片；(B) 油菜與芥藍種間雜交植株。

Fig. 3. An interspecific hybrid plant (*Brassica campestris* ssp. *chinensis* × *Brassica oleracea* var. *aboglabra*) (B). Note leaf morphology is different among the hybid plant (A, center) and its female parent, *B. campestris* (mock pak-choi) (A, left) and male parent, *B. oleracea* (Chinese kale) (A, right).

Wen *et al*. 2008a)。油菜與芥藍為蕓苔屬的不同種，分屬於AA與CC染色體組，藉由觀察雜交胚的發育過程得知，雜交胚約在授粉後第20日起雜交胚在外型上由綠色球型胚開始轉為淡褐扁平化終至褐化皺縮死亡，顯示芥藍與油菜種間雜交胚敗育發生在球型胚時期。授粉後子房發育時間的長短會影響內部胚珠的發育及存活率 (Kudou *et al*. 1995; Wang *et al*. 2008b)，在前人研究中，油菜 × 羽衣甘藍以授粉後7日齡子房培養其雜交株獲得率最高，但其反交羽衣甘藍 × 油菜僅能以授粉後16–18日齡胚培養獲得雜交株 (Wen *et al*. 2008a)；Zhang *et al*. (2004) 則認為*B. rapa* × *B. oleracea*以授粉後9日的子房為較適合的培養時期；Kudou *et al*. (1995) 以授粉後18–36日齡是青花菜與油菜雜交胚培養獲得很好的結果；其他研究報告也有相似的結論 (Lu *et al*. 2001; Man *et al*. 2007; Takeshita *et al*. 1980)。Zhu & Wei (2009) 曾報導*B. oleracea*與*B. campestris*種間雜交難以著果，並認為胚的皺縮可能由於胚珠組織的扁平化，不充足及有障礙的養分輸送可能為胚敗育的主因。雜交組合及親本基因型的差異會影響遠緣雜交親合性的強弱，因而影響雜交胚在受精後至敗育萎縮前發育期間的長短。我們的試驗結果則認為授粉後10–15日齡的未成熟胚是油菜與芥藍適當胚拯救培養時期，此結果與前人在菜心與芥藍雜交的培養結果相似 (Yang *et al*. 2008)；但與前述部分學者的研究結論不同 (Kudou *et al*. 1995; Lu *et al*. 2001; Man *et al*. 2007)。但是可以確定的是十字花科蔬菜遠緣雜交胚在早期即退化，而且認為適當摘取培養時間會依雜交組合、物種品系及植株生育狀況而有差異，因此遠緣雜交育種胚拯救時確認適當胚的發育期是非常重要的。

近年因技術不斷改良提升，遠緣雜種獲得率大幅提高，明顯地視親本組合、培養基、授粉母本基因型的影響 (Inomata 1978, 2007; Man *et al*. 2007; Wang *et al*. 2008a; Zhang *et al*. 2004)。種原間的親疏遠近對於遠緣雜交親本間的親和性具有決定性的影響，即使是相同基因型的兩個種原親本進行正交與反交其雜交胚培養成功率及生育反應並不完全相同，此種單方向不親和特性在蕓苔屬族群常見 (Bhat & Sarla 2004; Inomata 1978, 2007; Man *et al*. 2007; Wang *et al*. 2008a)。就蕓苔屬作物而言，子房培養明顯地可以獲得*B. rapa* × *B. oleracea*雜交

株，但是其反交則無效 (Huang *et al*. 2008; Inomata 1978; Man *et al*. 2007; Nishi *et al*. 1959)；但是Takeshita *et al*. (1980) 則認為經由胚培養可輕易獲得以*B. oleracea*為母本的種間雜交株。合成*B. juncea*時以*B. rapa*為母本獲得雜交株率較高，以*B. nigra*為母本的反交組合則有明顯的不親合性 (Bhat & Sarla 2004)。此試驗以芥藍與油菜進行正反交胚培養結果，以*B. campestris*為育種母本時雜交胚多直接萌發成植株，萌芽率較高；若以*B. oleracea*為母本之雜交組合僅少數培植體形成雜交單株，多數培植體趨向於形成愈合組織及根分化，後者可能與培養基組成不適合生長或是受育種母本基因型的影響，尚有待繼續探討。但是不同染色體數目的種間雜交時，採用染色體數目高的作母本較易成功，Stewart (2004) 也認為以*B. oleracea*為母本與*B. rapa*雜交胚培養成功率依然非常低；在前人芥菜型油菜與白菜、菜心與芥藍、甘藍與大白菜的種間雜交也得到相似的結論，並認為正反交差異可能受細胞質基因組的決定 (Man *et al*. 2007; Stewart 2004; Wang *et al*. 2006; Yang *et al*. 2008)；說明蕓苔屬種間雜交母本基因型不同，對雜種的誘導率有一定的影響。

培養基組成已被證實會影響多種作物胚拯救的存活率，進行雲苔屬蔬菜種間雜交胚培養，有些研究認為在不含生長調節劑的基本培養基未成熟胚培養都無法發育成苗 (Kudou *et al*. 1995)，但是我們以基本培養基培養油菜與芥藍仍然可以獲得少數的雜交株，相同結果亦見於油菜與蕓芥 (Dai *et al*. 2004)、羽衣甘藍與油菜 (Wen *et al*. 2008a) 等種間雜交株的培育，但若在培養基中添加適量的生長調節劑NAA及kinetin明顯地可促進*B. campestris*與*B. oleracea*種間雜交胚在試管內的發育，其他研究也獲得相似的結果 (Bennett *et. al*. 2008; Dai *et al*. 2004; Kudou *et al*. 1995; Wang *et al*. 2008a; Yang *et al*. 2008; Zhang *et al*. 2004)。另外，培養基組成中鹽類濃度含量會影響受精胚的發育，Inomata (1979) 曾提出二倍體植株子房內種子形成時需要較少的硝酸鹽含量，不同基因型或雜交組合的雜交胚對無機鹽類的需求不同。此試驗中以全量MS配方為基本培養基，在相同的添加物中以*B. oleracea*為母本的組合雜交株獲得率較較其反交組合差且培植體生育反應偏向根分化，可能因授粉母本不同對鹽類或滲透壓需求並不相同，此推測尚有待進一步的實驗證實。

經由胚培養獲得之培植體，需進一步鑑定是否確為種間雜交之後裔，通常可利用顯微鏡進行染色體數判斷、核型分析或以螢光原位雜交 (FISH) 等鑑定之，然而若為育種之目的進行大量種間雜交試驗時，快速有效的種間雜種鑑別方法將有助於提升篩選效率，本試驗以流式細胞儀分析雜交組合後裔與親本間之相對DNA含量可清楚鑑別出種間雜交株，Fahleson *et al*. (1988) 即以流式細胞儀分析十字花科作物中甘藍與白菜的體細胞雜種之核酸含量，當個體間核酸含量具一定之差異，以該法測定之DNA含量與染色體數目之相關係數達0.91，用之估計雜種染色體數的誤差約在±10%左右，隨後亦有研究以該法分析甘藍類之體細胞雜種 (Sheng *et al*. 2008)，更甚者以該法分析花椰菜與黑芥之非對稱融合體細胞雜種後裔的相對DNA含量變異，並藉其證明個體間染色體丟失之程度 (Zhang *et al*. 2008)。本試驗以流式細胞儀分析雜交組合後裔與親本間之相對DNA含量，該種間雜種的相對DNA含量介於雙親之間且趨近於雙親之平均值，此與南瓜屬種間雜交之結果雷同 (Šiško *et al*. 2003)，相對於前述體細胞非對稱融合而言，種間雜交胚較無因染色體丟失造成之染色體數目變異大的情形，因此更能清楚鑑別出種間雜交株。

Sabharwal & Doležel (1993) 應用流式細胞

儀分析芸苔屬種間雜交植株倍數性及基因組 (genome) 之組成時指出，CC基因組比AA基因組多了約44%的核酸含量，而本試驗中屬CC基因組之芥藍‘1508’其相對DNA含量約比AA基因組之油菜‘B-03’多了約52–54% (G2與G1 stage)，CC基因組核酸含量明顯大於AA基因組的結果類似，其DNA含量數值之差異可能為核酸染劑不同或試驗材料差異所致。此外，由於CC基因組與AA基因組間核酸含量差異夠大，即使流式細胞儀讀值與染色體數間雖可能有誤差存在，仍能有效區別種間雜種。若為育種之目的而進行回交時，由於染色體之不對等分離將使後裔個體間染色體數有較大之變異，則可能需配合其他方法輔助篩選種間雜交後裔個體。

雜交後裔特性偏雌效應曾經在不同作物發現，在芸苔屬中如*B. napus*油含量、種子種皮色素遺傳等性狀會受授粉母本細胞質影響等 (Bhat & Sarla 2004; Huang *et al*. 2008; Kondra & Stefansson 1970; Nishi *et al*. 1959; Tian *et al*. 2010; Wang *et al*. 2008a; Wen *et al*. 2008a)。此試驗油菜與芥種間雜交實生後代園藝性狀雖然介於雙親之間，但是由3組正反交實生苗外表型態比較，以*B. oleracea*為授粉母本則雜交株的葉片略帶革質、葉色呈現暗綠，以*B. campestris*為母本則雜交株葉片質地較薄、葉色較淺綠，也有偏母本特性的趨勢，與前人的研究結果相符。此試驗中不同品系芥藍與油菜正反交結果，8個雜交組合中僅油菜‘B-01’ × 芥藍‘1508’雜交株具有少量花粉，其餘7個組合均為不稔的雜交株。遠緣雜交株普遍有不稔性的障礙，前人研究認為遠緣雜交株在減數分裂時可能有染色體數分配不均勻及染色體丟失干擾花粉正常基因功能 (Huang *et al*. 2008; Tian *et al*. 2010; Wang *et al*. 2008a, 2008b)，利用染色體倍加技術提高遠緣雜交株的稔實性目前已有初步成果，將近一步探討遠緣雜交種後裔表現。

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Identification of Interspecific Hybrids from Crosses between *Brassica oleracea* and *B. campestris*[1]

Chin-Chun Chiou[2], Tzu-Kai Lin[3,5], Chen-Yu Lin[2], Chi-Ni Hsia[4], and San-Tai Wang[2]

Abstract

Chiou, C. C., T. K. Lin, C. Y. Lin, C. N. Hsia, and S. T. Wang. 2012. Identification of interspecific hybrids from crosses between *Brassica oleracea* and *B. campestris*. J. Taiwan Agric. Res. 61:52–63.

In some crops, inter-specific hybridization is more useful than intra-specific hybridization for improvement of crop yield and quality. The objective of this study was to develop interspecific hybrids using different lines of *Brassica oleracea* var. *aboglabra* and *B. campestris* ssp. *chinensis*. After reciprocal bud pollination, imature hybid embryos were collected and cultured on the modified Murashige and Skoog's medium. Results showed that the efficiency of producing interspecific hybrids was affected by embryo stage, genotype and medium composition. The efficiency of embryo rescue was affected by genotype and more interspecific hybrids were produced when *B. campestris* was used as female. The phenotype and relative amount of DNA in hybrids were between their parents but these characteristics were affected more by female parent than male parent.

Key words: *Brassica oleracea* var. *alboglabra*, *Brassica campestris* ssp. *chinensis*, Interspecific hybridization, Embryo rescue.

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台灣農業研究 (J. Taiwan Agric. Res.) 61(1):64–74 (2012)

Identification of Capsicum Chlorosis Virus Causing Chlorotic Spots and Stripes on Calla Lily[1]

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Abstract

Chen, T. C., C. A. Chang, Y. C. Kang, S. D. Yeh, C. H. Huang, and C. C. Chen. 2012. Identification of Capsicum chlorosis virus causing chlorotic spots and stripes on calla lily. J. Taiwan Agric. Res. 61:64–74.

Calla lily (*Zantedeschia* spp.) is one of the economically important ornamental crops in Taiwan. In a field survey of calla lily conducted during 2005, plants showing symptoms of yellow spots and stripes on leaves were observed in Houli Township, one of the major areas for commercial production of ornamental crops in Taiwan. Fifteen virus isolates were collected from diseased plants of calla lily and purified via three successive local-lesion isolations on leaves of inoculated *Chenopodium quinoa*. A 0.9 kb DNA fragment was amplified from total RNA extracted from all the fifteen virus isolates on infected plants by reverse transcription-polymerase chain reaction (RT-PCR) using the *Tospovirus* genus-degenerate primers gL3637 and gL4510c, designed from the conserved regions of L RNA, revealing that the disease was caused by a *Tospovirus*. The virus isolates reacted positively with the antiserum to the nucleocapsid (N) protein of Capsicum chlorosis virus (CaCV) and the monoclonal antibody to the N protein of *Watermelon silver mottle virus* (WSMoV), indicating that they are members of the WSMoV serogroup. The nucleotide sequences of the N gene of these virus isolates from calla lily were phylogenetically related to CaCV. Furthermore, the pathogenicity of CaCV was also verified by inoculation tests on plants of calla lily.

Key words: Virus disease of calla lily, *Tospovirus*, Capsicum chlorosis virus (CaCV).

Introduction

Calla lily (*Zantedeschia* spp.), belonging to *Araceae*, is a popular ornamental crop in Taiwan and many other countries. Nine viruses have been reported as causal agents of calla lily in Taiwan, including *Calla lily latent virus* (CLLV) (Chen *et al.* 2006b, 2006c), *Dasheen mosaic virus* (DsMV) (Zettler & Hartman 1987, 1995), *Konjak mosaic vi-*

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rus (KoMV) (Pham *et al.* 2002), *Turnip mosaic virus* (TuMV) (Chen *et al.* 2003) and *Zantedeschia mild mosaic virus* (ZaMMV) (Chen *et al.* 2006c; Huang & Chang 2005) of the genus *Potyvirus*; *Cucumber mosaic virus* (CMV) (Zettler & Hartman 1995) of *Cucumovirus*, *Carnation mottle virus* (CarMV) (Chen *et al.* 2002) of *Carmovirus*, *Lisianthus necrosis virus* (LNV) (Chen *et al.* 2006d) of *Tombusvirus*; and Calla lily chlorotic spot virus (CCSV) (Chen *et al.* 2005; Lin *et al.* 2005) of *Tospovirus*.

The genus *Tospovirus*, transmitted by thrips in a persistent manner, is the only plant-infecting genus in the family *Bunyaviridae* (Fauquet *et al.* 2005). The enveloped quasi-spherical particles of tospoviruses are 80 to 110 nm in diameter and contain a tripartite single-stranded (ss) RNA genome denoted large (L), middle (M) and small (S) segments (Fauquet *et al.* 2005). The negative sense L RNA encodes a large RNA-dependent RNA polymerase in the viral complementary (vc) strand for virus replication (de Haan *et al.* 1990). Both M- and S-RNAs are ambisense and each contains two open reading frames (ORFs). The viral (v) strand of M-RNA encodes a nonstructural (NSm) protein for cell-to-cell movement of non-enveloped ribonucleocapsid structures (Kormelink *et al.* 1994; Lewandowski & Adkins 2005), while its vc strand encodes the precursor of Gn and Gc glycoproteins for composing spikes on the viral envelope (Kormelink *et al.* 1992; Law *et al.* 1992). Another nonstructural (NSs) protein encoded by the v strand of S RNA is a gene-silencing suppressor responsible for counteracting the defense mechanisms in plants (Bucher *et al.* 2003; Takeda *et al.* 2002), and forms filamentous inclusion bodies in the infected cells (Kormelink *et al.* 1991). The vc strand of S-RNA encodes the nucleocapsid (N) protein for encapsidation of viral RNAs (de Haan *et al.* 1990).

A threshold of 90% amino acid identity in N protein is the key criterion for demarcation of tospoviruses at species level (Goldbach & Kuo 1996). In addition, tospoviruses can be clustered in serogroups or classified as serotypes on the basis of the serological and phylogenetic relationships of N proteins (Adam *et al.* 1993). The serological-related viruses are grouped as a serogroup, and viruses without serological relationship are designated as monospecies serotypes. So far, there are 22 formal and tentative tospovirus species that have been characterized (Seepiban *et al.* 2011; Zhou *et al.* 2011). Most of them were claded into three major serogroups designating from type members, *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV) and Iris yellow spot virus (IYSV). Three tospovirus species, *Impatiens necrotic spot virus* (INSV), *Peanut yellow spot virus* (PYSV) and Peanut chlorotic fan-spot virus (PCFV), were classified as monospecies serotypes (Chen *et al.* 2010).

Capsicum chlorosis virus (CaCV), a member of WSMoV serogroup, was first found to infect capsicum and tomato in Queensland, Australia (McMichael *et al.* 2002) and further recognized as a widespread pathogen in solanaceous crops in Thailand (Chiemsombat *et al.* 2008; Knierim *et al.* 2006). Recently, it has become an important quarantine virus for the production of *Phalaenopsis* orchids in Taiwan (Zheng *et al.* 2008). In 2005, symptoms of yellow spots and stripes on leaves of calla lily plants were found in cultivated fields in Houli Township, Taiwan. Fifteen virus isolates were collected from diseased plants by single lesion isolations. All fifteen virus isolates were further identified as isolates of CaCV by sequence determination of their N genes (Chen *et al.* 2007a). In this study, one of calla lily isolates of CaCV (FG1) was back inoculated to calla lily plants to verify that CaCV is the natural causal agent of chlorotic spots and stripes of calla lily. The molecular relationships of the calla lily isolates of CaCV were also analyzed.

Materials and Methods

Virus inoculation

Fifteen isolates of CaCV collected from calla lily in Houli Township, Taichung, Taiwan in 2005 were previously described by Chen *et al.* (2007a). Other viruses used in this study were: one isolate of CaCV from gloxinia, denoted HT-1, which was collected in the United States by Dr. H.-T. Hsu (Hsu *et al.* 2000) and one isolate of WSMoV from watermelon (Yeh *et al.* 1992) and one isolate of CCSV from calla lily (Chen *et al.* 2005) in Taiwan. All tospoviruses were maintained on leaves of *Chenopodium quinoa* and *Nicotiana benthamiana* by mechanical inoculation. A calla lily isolate of CaCV, denoted FG1, was mechanically inoculated on leaves of calla lily seedlings (*Zantedeschia elliottiana* cv. 'Pot of

Gold'). The virus-infected tissues were ground with 0.05 M potassium phosphate buffer (pH 7.5) in a ratio of 1:10 (w/v) and the crude saps were introduced onto leaves of calla lily plants. The inoculated plants were kept in a screenhouse used for further serological and molecular assays.

Indirect enzyme-linked immunosorbent assay (ELISA)

The procedures of indirect ELISA were conducted as previously described (Chen *et al.* 2003; Clark & Adams 1977). The rabbit antisera to the calla lily-infecting viruses were prepared in our laboratory and used for serological tests of viruses, including CarMV, CMV, CLLV, DsMV, KoMV, TuMV and ZaMMV (Chen *et al.* 2006a, 2006c; Chen *et al.* 2007a), the antiserum against the N protein of CaCV HT-1 (Hsu *et al.* 2000), the monoclonal antibodies (MAbs) against the N protein of CCSV and WSMoV (Lin *et al.* 2005) and the *Potyvirus*-general MAb (Agdia, Elkhart, IN). The alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG and the AP-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) were used at a 1:5000 dilution as the secondary antibodies for detecting rabbit and mouse antibodies, respectively. Absorbance at 405 nm (A_{405}) was recorded by PTI max microplate reader (Molecular Devices, Sunnyvale, CA) 30 min after the addition of ρ-nitrophenyl phosphate substrate (1 mg/mL) (Amresco, Solon, OH).

Cloning and sequencing

Extraction of total RNAs of virus-infected plant tissues using the Plant Total RNA Miniprep Purification Kit (Hopegen, Taichung, Taiwan), and reverse transcription (RT) of the first strand cDNA were conducted as described previously (Chen *et al.* 2006b). The primer pairs, gL3637 [5′-CCTTTAACAGT(A/T/G)GAAACAT-3′]/gL4510c [5′-TCATC(A/G)GA(A/G)TG(T/G/C)AC(A/C)ATCCATCT-3′] (Chu *et al.* 2001), designed from the conserved regions of tospoviral L RNAs, and WN2328 (5′-CCATTGGTTTGCCTCCG-3′)/WN3534 (5′-CGTCGACAGAGCAATCGAGGC-3′) (Chen *et al.* 2007a), designed from the S RNA of WSMoV, were used for amplification of a partial L gene fragment and a full-length N gene, respectively. Polymerase chain reaction (PCR) amplification was carried out by 26 cycles: denaturing at 94°C for 1 min, annealing at 50°C for 45 sec, and DNA synthesis at 72°C for 90 sec. An elongation step at 72°C for 6 min was conducted at the last additional cycle. Amplified DNA products were analyzed by electrophoresis in a 1% agarose gel. The amplified DNA fragments corresponding to the full-length N genes were cloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. Nucleotide sequences were determined by an automatic DNA sequencer (ABI PRISM 377, Perkin-Elmer, Foster City, CA). Three independent clones were selected for alignment to determine the correction of nucleotide sequences.

Sequence and phylogenetic analyses

Prediction of open reading frames (ORFs) in the determined sequences was conducted using the ORF program of Vector NTI (Invitrogen). Multiple sequence alignments were carried out by the Clustal W program of Vector NTI. The nucleotide sequences were translated into deduced amino acid sequences using the Translate Selection program of Vector NTI. The amino acid sequence alignments were performed by the Align X program of Vector NTI. The phylogenetic relationships among different virus isolates, according to the complete amino acid sequences of N proteins, were also analyzed. The N protein sequences of WSMoV (accession no. U78734), CaCV (accession no. AY036057), CCSV (accession no. AY867502), TSWV (accession no. D13926), INSV (accession no. X66972), and PCFV (accession no. AF080526) were used for comparison. Analyzed sequences were first aligned using Clustal X version 1.8 (Jeanmougin *et al.* 1998). Their phylogenetic relationships were determined using PAUP 4.0 (Swofford, 1998) by the Neighbour-Joining algorithm with the bootstrap resampling method (Felsenstein 1985; Thompson *et al.* 1997). One thousand random resamplings were used to calculate the bootstrap values. The calculated trees were displayed by the TreeView program (Page 1996).

Primer design for virus identification

Specific primers, CaCV4f (5′-TCTACCGTCAGGCAACTTACCGAG-3′) and CaCV777c (5′-ATAATCATCCACAGACAAATTGGCACTAAA-3′), were designed to identify CaCV from the infected plant tissues. RT-PCR was conducted by One-Step RT-PCR Kit (Hopegen, Taichung, Taiwan) as the manufacturer's instruction. The first strand

cDNAs were synthesized at 50°C for 30 min and terminated at 94°C for 2 min, and then PCR was performed by 35 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min. The amplicons were analyzed by electrophoresis in 1% agarose gels.

Results

Isolation and identification of virus from calla lily in Taiwan

In 2005, symptoms of chlorotic spots and stripes accompanying necrosis on leaves of calla lily plants were found in cultivated fields in Houli Township, Taiwan (Fig. 1A). Fifteen symptomatic calla lily samples were collected from field and tested in indirect ELISA. All of the tested samples did not react with the *Potyvirus*-general MAb (Agdia) and the antisera to CarMV, CLLV, CMV, DsMV, KoMV, TuMV and ZaMMV, which are common calla lily-infecting viruses (Chen *et al.* 2003, 2006b; Huang & Chang 2005; Zettler & Hartman 1995). The *Tospovirus* genus-degenerate primers gL3637 and gL4510c (Chu *et al.* 2001) were used in RT-PCR to amplify a 0.9-kb DNA fragment from all samples (Fig. 2). Thus, the calla lily-infecting virus isolates were predicted as tospoviruses. Furthermore, the antiserum to CaCV HT-1 (denoted RAs-CaCV) and the MAbs to CCSV and WSMoV were used in indirect ELISA to verify these calla lily virus isolates. All samples were positively reacted with RAs-CaCV and the MAb to WSMoV (denoted MAb-WSMoV), but not reacted with MAb to CCSV (data not shown). The results demonstrated that the diseased calla lily samples collected from Houli Township were infected by a *Tospovirus* of WSMoV serogroup.

Isolation and back-inoculation of calla lily-infecting tospoviruses

Fifteen virus isolates designated as BM11, BM12, BM13, BM14, BM17, FG1, FG2, FG3, FG13, PG5, PG22, PG23, PG25, PG28 and PG30, were obtained from successive single-lesion isolations on inoculated chenopodium plants. All virus isolates produced similar necrotic local lesions on the inoculated leaves of *C. quinoa* plants (Fig. 1B) and they produced typical symptoms of leaf curl and mosaic on leaves of inoculated plants of *N. benthamiana* (Fig. 1C). The individual virus isolates were confirmed by positively reacting with RAs-CaCV and MAb-WSMoV in indirect ELISA (data not shown). The FG1 isolate was mechanically introduced to ten calla lily seedlings and the results showed that one of the inoculated seedlings showed yellow spots on the newly extended leaf (Fig. 1D). An average ELISA reading of 0.38, two-fold higher than the reading of negative control (0.08), was obtained when the symptomatic calla lily tissue reacted with RAs-CaCV in indirect ELISA to confirm the CaCV infection.

Sequence and phylogenetic analyses of the N genes of the calla lily isolates of CaCV

The primers WN2328 and WN3534, designed from the intergenic region and the 3′-untranslatable region of the S-RNA of WSMoV, respectively, were used to amplify a 1.1-kb DNA fragment from total RNA extract of the aforementioned CaCV isolates. Sequence determination revealed that the amplified DNA fragments correspond to full-length N genes. The N gene sequences of the fifteen virus isolates were submitted to GenBank to obtain accession numbers (Table 1). The N genes of all fifteen CaCV isolates share a high homology of 95.8–97.1% nucleotide sequence identities and 97.5–98.2% amino acid sequence identities with that of the typical CaCV Australian isolate (accession no. AY036057), but a lower homology of 76.7–78.1% nt identities and 84.4–86.2% aa identities with that of the original WSMoV isolate from Taiwan (accession no. U78734). Phylogenetic analysis of the N proteins of fifteen calla lily-infecting CaCV isolates and other tospoviruses revealed that these isolates of CaCV can be divided into two groups: isolates BM17, FG1, FG3, FG13, PG23, PG28 and PG30 were closely related to HT-1 and were clustered as a group, while isolates BM11, BM12, BM13, BM14, FG2, PG5, PG22 and PG25 were clustered as another group (Fig. 3).

Identification of CaCV by RT-PCR

A 0.77-kb DNA fragment was amplified from all tested samples of CaCV isolates by RT-PCR using the primers CaCV4f and CaCV777c, but no signals were obtained from WSMoV (Fig. 4). The results indicated that CaCV can be distinguished from WSMoV by the newly designed specific primers in RT-PCR.

Discussion

In addition to previous reports of *Tospovirus*, such as TSWV (Zettler & Hartman 1995) and CCSV

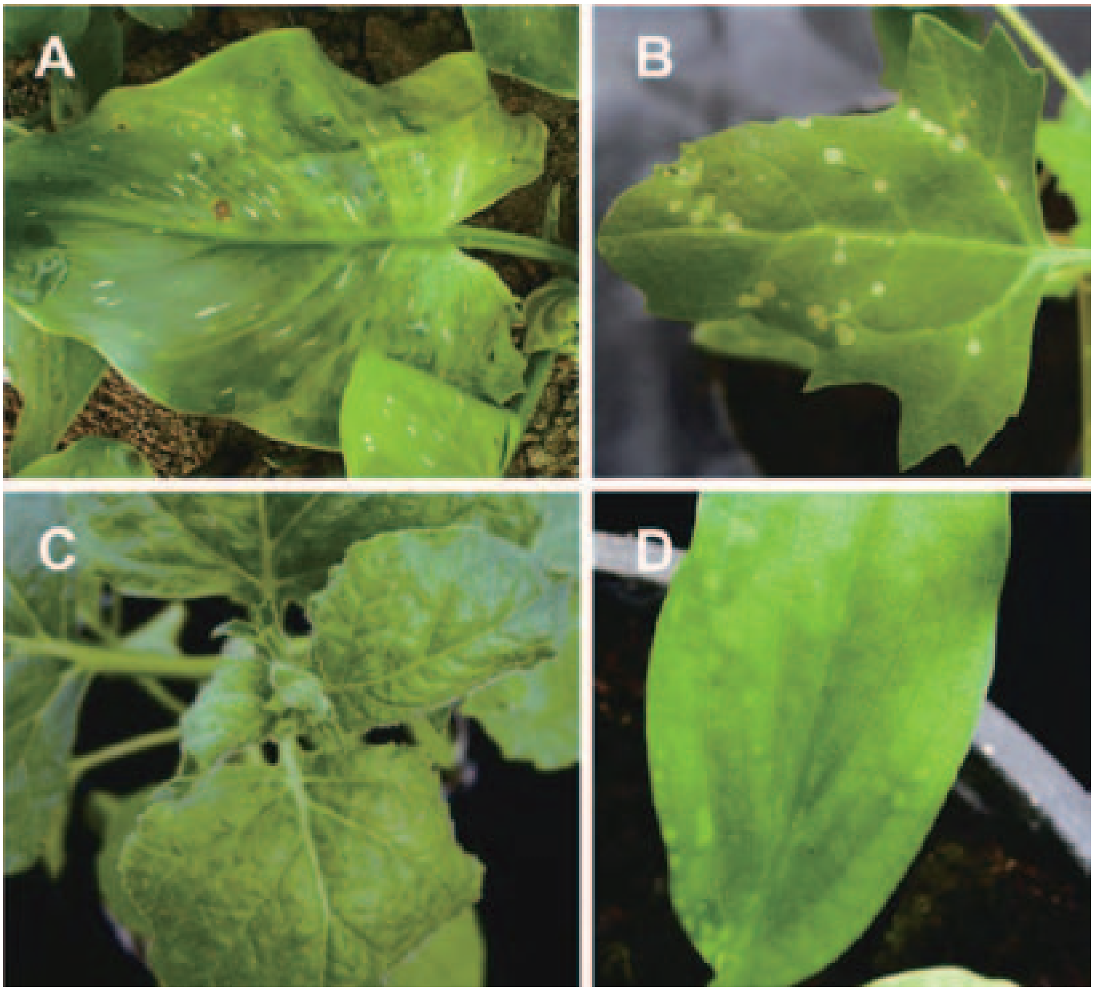

Fig. 1. Symptoms of calla lily-infecting virus isolates on calla lily (A, D), *Chenopodium quinoa* (B) and *Nicotiana benthamiana* (C). (A) Symptoms of yellow spots and stripes on a leaf of a calla lily plant grown in the field; (B) Local lesions produced on a leaf of *Chenopodium quinoa* inoculated with a calla lily-infecting virus isolate FG1; (C) *Tospovirus*-caused chlorotic spot symptoms were observed on the leaves of *Nicotiana benthamiana* inoculated with a single-lesion isolated virus isolate FG1; and (D) Yellow spots produced on a systemic leaf of calla lily inoculated with the FG1 isolate after inoculation for 21 days.

Fig. 2. Detection of fifteen calla lily-infecting virus isolates by reverse transcription-polymerase chain reaction. The degenerate primer pair gL3637/gL4510c (Chu *et al.* 2001), designed from the consensus sequences of tospoviral L RNAs, was used to amplify a 0.9-kb DNA fragment from total RNAs extracted from the naturally infected calla lily samples. Lane M, 100 ladder marker; lane 1 to 15, virus isolates BM11, BM12, BM13, BM14, BM17; FG1, FG2, FG3, FG13; PG5, PG22, PG23, PG25, PG28 and PG30; lane C, Capsicum chlorosis virus (CaCV) gloxinia isolate HT-1; lane W, *Watermelon silver mottle virus* (WSMoV); and lane H, healthy calla lily as negative control.

Table 1. Nucleotide (nt) and amino acid (aa) identities of the nucleocapsid genes of the calla lily-infecting tospovirus isolates compared with those of the typical isolates of Capsicum chlorosis virus (CaCV) and *Watermelon silver mottle virus* (WSMoV)

		Identity (%) to			
		CaCV [z]		WSMoV	
Virus isolate	Accession no.	nt	aa	nt	aa
BM11	EF100597	96.6	97.5	77.7	85.5
BM12	EF095725	95.8	97.5	76.7	84.4
BM13	EF095727	97.0	97.8	78.1	85.8
BM14	EF095726	96.8	98.2	78.1	86.2
BM17	EF100595	96.5	98.2	77.5	85.1
FG1	EF100598	96.4	97.8	77.5	85.5
FG2	EF100600	97.1	97.8	77.9	85.8
FG3	EF100601	96.1	97.5	77.3	85.1
FG13	EF100599	96.2	97.5	77.5	85.5
PG5	EF100596	97.1	97.8	77.9	85.8
PG22	EF100602	96.6	97.8	77.9	85.8
PG23	EF100603	96.4	97.8	77.5	85.1
PG25	EF100604	96.6	97.8	77.9	85.8
PG28	EF100605	96.8	97.8	77.9	85.8
PG30	EF100606	96.0	97.5	77.3	85.1

[z] Accession no. AY036057 for CaCV and accession no. U78734 for WSMoV were used for sequence comparisons.

(Lin *et al.* 2005) on calla lily, this study reveals another *Tospovirus* CaCV responsible for the disease of calla lily occurred in Houli (Taichung, Taiwan) in 2005 was verified to invade calla lily naturally. Diseased calla lily plants infected with CaCV did not mix-infected with other viruses were confirmed by serological tests using various antisera and MAbs to calla lily-infecting viruses and bioassays via single-lesion isolation. Symptoms caused by various viruses on calla lily plants in Taiwan were similar. For instance, symptoms caused by CaCV are similar to those induced by TuMV and CCSV, showing green, yellow or chlorotic spots and strips on the infected leaves of calla lily plants (Chen *et al.* 2003, 2005). For this reason, diagnosis of calla lily virus diseases based on symptoms alone is difficult and unreliable.

Serological and molecular analyses for identification of calla lily-infecting viruses are recommended. Genus broad-spectrum degenerate primers and antibodies are effective tools for preliminary diagnosis of virus diseases in the field. In this study, field samples of diseased calla lily were successfully identified as tospovirus infections by RT-PCR using the *Tospovirus* genus-degenerate primer pair gL3637/gL4510c, which was designed from the consensus sequences of tospoviral L RNAs (Chu *et al.* 2001). It leaded a correct direction to diagnose a virus disease. CaCV was first identified in Australia as a pathogen of *Capsicum* spp. causing chlorotic spots on leaves (McMichael *et al.* 2002), and it was subsequently reported on solanaceous crops in Thailand (Chiemsombat *et al.* 2008) and India (Kunkalikar *et al.* 2010) and on peanut (*Arachis hypogaea* L.) in China (Chen *et al.* 2007b) and Thailand (Chiemsombat *et al.* 2008). In Taiwan, CaCV was reported as a pathogen mainly on tomato (Huang *et al.* 2010) and ornamental crops, such as orchids (Zheng *et al.* 2008), calla lily (Chen *et al.* 2007a), amaryllis (*Hippeastrum hybridum* Hort.) and blood lily (*Haemanthus multiflorus* Martyn.) (Chen *et al.* 2009).

CaCV is genomically closely related to WSMoV with homologies of 84–91% amino acid sequence identities in each viral protein (Knierim *et al.* 2006), and they are serologically indistinguish-

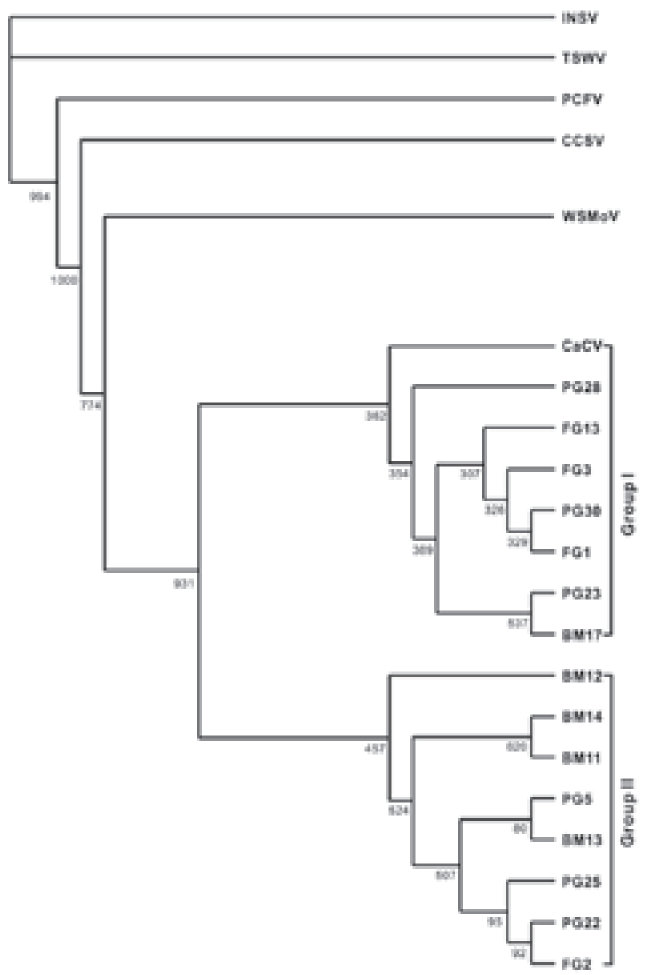

Fig. 3. Phylogenetic analysis of the nucleocapsid (N) proteins of fifteen calla lily-infecting virus isolates and other tospoviruses. The tree was constructed using the neighbour-joining algorithm with the bootstrap resampling method (1000 random resamplings) using PAUP 4.0. Accession numbers of the N protein sequences of the individual virus isolates are listed in Table 1. The N protein sequences of the typical isolates of Capsicum chlorosis virus (CaCV; AY036057), *Watermelon silver mottle virus* (WSMoV; U78734), *Calla lily chlorotic spot virus* (CCSV; AY867502), *Tomato spotted wilt virus* (TSWV; D13926), *Impatiens necrotic spot virus* (INSV; X66972) and *Peanut chlorotic fan-spot virus* (PCFV; AF080526) are used for comparison.

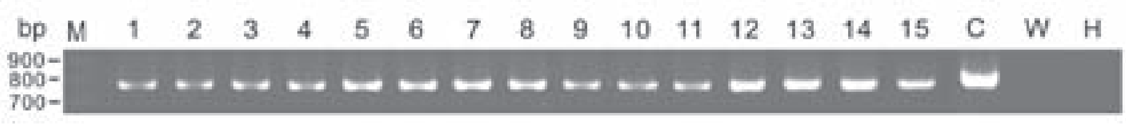

Fig. 4. Design of specific primers for identification of Capsicum chlorosis virus (CaCV) infecting calla lily in reverse transcription-polymerase chain reaction. The primers CaCV4f and CaCV777c were designed to amplify a 0.77-kb DNA fragment from CaCV-infected samples. Lane M, 100 ladder marker; lane 1 to 15, virus isolates BM11, BM12, BM13, BM14, BM17; FG1, FG2, FG3, FG13; PG5, PG22, PG23, PG25, PG28 and PG30; lane C, CaCV gloxinia isolate HT-1; lane W, *Watermelon silver mottle virus* (WSMoV); and lane H, healthy calla lily.

able on the basis of N and NSs proteins (Chen *et al.* 2010). Our results also revealed that CaCV and WSMoV are indistinguishable by RT-PCR analysis using the WSMoV N gene primers WN2328 and WN3534, which were designed for specifically for detection of WSMoV in field survey (Chen *et al.* 2007a). Previous reports also indicate that CaCV, WSMoV, PBNV and WBNV are the four closely related WSMoV-serogroup tospoviruses and are difficult to distinguish by serological assays (Chen *et al.* 2005; Jain *et al.* 2007; Zheng *et al.* 2008). Thus, determination of the full-length N gene sequences is necessary for verifying these tospoviruses.

Development of highly specific primers for RT-PCR amplification is important for detection and identification of viruses. A previously designed primer pair WN2963/WN3469c was reported to identify WSMoV from field samples (Chen *et al.* 2010). Here, a CaCV-specific primer pair CaCV4f/CaCV777c was designed and successfully used in RT-PCR to differentiate CaCV from WSMoV (Fig. 4). These two primer pairs are useful and convenient tools to detect and differentiate CaCV and WSMoV in the fields. In addition to further differentiate the four serologically close-related tospoviruses, such as WSMoV, CaCV, PBNV and WBNV, a convenient one tube-based multiplex RT-PCR method using the combination of individual virus-specific primer pairs will be developed.

No insect vectors were found in the diseased calla lily fields. To our knowledge, WSMoV can be transmitted by *Thrips palmi*, which is a common thrips in Taiwan (Chen *et al.* 1990). However, the reported vector of CaCV, such as *Ceratothripoides claratris* (Premachandra *et al.* 2005), was not found in Taiwan. The category of thrips transmitting CaCV in field should be investigated.

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Capsicum Chlorosis Virus 引起彩色海芋黃化斑點及條斑之鑑定[1]

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摘 要

陳宗祺、張清安、康雅琪、葉錫東、黃春惠、陳金枝。2012。Capsicum chlorosis virus 引起彩色海芋黃化斑點及條斑之鑑定。台灣農業研究 61:64–74。

彩色海芋 (*Zantedeschia* spp.) 是國內重要之經濟花卉，本研究於2005年在后里地區觀察到彩色海芋植株葉片出現黃色斑點及條斑病徵。取不同罹病植株病葉經由連續三次單斑接種於奎藜 (*Chenopodium quinoa*) 後，共獲得15個純系之病毒分離株。此等病毒分離株之核酸以對應*Tospovirus* L基因之廣效性引子對gL3637/gL4435c進行反轉錄-聚合酶鏈鎖反應 (Reverse-transcription polymerase chain reaction, RT-PCR)，均可獲得與預估相符約0.9 kb之DNA片段，顯示分離株均為*Tospovirus*屬病毒之成員。而分離株所產生之單斑均與對應核鞘蛋白 (nucleocapsid protein, NP) 之抗血清包括番椒黃化病毒 (Capsicum chlorosis virus, CaCV) 多元抗體及西瓜銀斑病毒 (*Watermelon silver mottle virus*, WSMoV) 單株抗體產生正反應，顯示其為*Tospovirus*屬WSMoV血清群之成員。經全長核鞘蛋白基因序列及類緣演化分析結果，顯示此等病毒均為CaCV的分離株。進一步完成彩色海芋寄主之回接試驗，病毒分離株可於植株葉片造成與田間觀察到之類似的黃斑病徵，證實CaCV為引起此病害之病毒。

關鍵詞：彩色海芋病毒病、*Tospovirus*屬病毒、番椒黃化病毒。

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雙條長葉蚤 (鞘翅目：金花蟲科) 之發生與防治長梗滿天星之潛力 [1]

王清玲 [2,4] 謝玉貞 [3] 林鳳琪 [2] 蔣慕琰 [3]

摘　要

王清玲、謝玉貞、林鳳琪、蔣慕琰。2012。雙條長葉蚤 (鞘翅目：金花蟲科) 之發生與防治長梗滿天星之潛力。台灣農業研究 61:75–79。

雙條長葉蚤 (*Agasicles hygrophila* Selman & Vogt) 屬於鞘翅目金花蟲科，單食於莧科雜草長梗滿天星 [*Alternanthera philoxeroides* (Mart.) Griseb.]，是世界性雜草生物防治工具。台灣有本種葉蚤發生，於26–27℃、24 L養蟲環境下，卵期約5日；幼蟲期平均12–13日；蛹期4–5日；雌成蟲壽命可達50日以上，平均25.3日，一生平均產卵258粒。田野調查顯示本蟲局部發生於水生長梗滿天星，於春、秋季節密度較高，長梗滿天星被害後整片枯死；夏季高溫時葉蚤生長繁殖力降低，此時長梗滿天星則生長旺盛，由於本葉蚤與滿天星的最適生長溫度不同，自然發生的葉蚤雖會降低植株生勢，但對於長梗滿天星較難產生高度防治效果。在有特殊需要之情況下，可考慮人為飼養釋放本蟲，以擴增田間密度，達到防治目的。另行引入耐高溫環境之蟲系，亦有可能改變目前自然發生的葉蚤與長梗滿天星間之關係。

關鍵詞：雙條長葉蚤、長梗滿天星、台灣、生物防治。

雙條長葉蚤 (*Agasicles hygrophila* Selman & Vogt) 屬鞘翅目 (Coleoptera) 金花蟲科 (Chrysomelidae)，單食性，以長梗滿天星 [*Alternanthera philoxeroides* (Mart.) Griseb.] 為寄主，為其重要取食者。長梗滿天星為莧科 (Amaranthaceae) 植物，原產南美洲，目前已擴散蔓延至全球三十多個國家和地區，成為嚴重雜草。美國1963年自阿根廷引進本種葉蚤，次年開始釋放於阻塞水道與河川的長梗滿天星，數年內在佛羅里達以及東南部各州建立族群，且均有良好的防治效果 (Spencer & Coulson 1976)，成為水生雜草生物防治成功的著名案例；其後澳洲於1976年、紐西蘭於1982年、中國大陸於1987年均曾自美國引入雙條長葉蚤 (Julien & Griffiths 1998)，使本蟲成為雜草生物防治的代表性昆蟲之一 (Forno & Julien 2000)。

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台灣於1930年代已有長梗滿天星發生之記載 (Wu *et al.* 2004)，但何時進入台灣並沒有正式紀錄，本種葉蚤資料甚為缺乏，簡單的文字描述僅見於台灣產金花蟲科圖誌 (Lee & Cheng 2007)。本文報告雙條長葉蚤外形、習性、發育、繁殖，及其與寄主植物之分布等，並據以討論防治長梗滿天星之潛力。

室內試驗是利用本所繁殖小黑花椿象等天敵昆蟲之養蟲室，在26–27℃溫度以及24小時全光照下進行。試驗使用之蟲源採集自野外長梗滿天星，置於紗網罩內以盆栽長梗滿天星飼養。觀察時從卵開始個別置於小玻璃瓶 (6 cm × 3 cm = H × D) 內，幼蟲以截成3 cm長之一段帶葉長梗滿天星餵食，每日更換葉片，觀察外形、習性，記錄幼蟲生長發育、化蛹及羽化情形，觀察成蟲壽命與每日產卵數。

本種葉蚤成蟲體長5–6 mm，寬約2 mm，體表光澤，頭與胸黑色，翅鞘有明顯黑黃相間條紋，背中央黑色，左右翅鞘各有一條縱向不規則黑色區，餘黃色 (圖1A)。雌蟲產卵堆於葉片背面，一卵堆約十餘粒至三、四十粒，卵粒呈乳黃色長橢圓形，長約1 mm，排成兩列 (圖1B)。幼蟲共分三齡，頭部黑色，體背灰黃色，三對足，將化蛹的老熟幼蟲體色深黑，長約6 mm (圖1C)。化蛹時幼蟲會找一隱蔽處所，吐絲捲起葉片在內化蛹；或以口器齧食莖部造成侵入孔，然後潛入莖內化蛹，裸蛹淺黃色 (圖1D)。成蟲羽化後咬孔鑽出，具飛行與跳躍能力。雄蟲個體較雌蟲小，且腹部腹面末端稍微凹陷，不如雌蟲飽滿。

卵期相當一致，經4–5日孵化，平均4.9日 (表1)。幼蟲發育期長短在個體間頗有差異，尤以第二、三齡幼蟲，發育期間長短不一，第二齡自2–5日，第三齡自3–10日均有，整個幼蟲期需時11–18日不等，平均12.3日。蛹期4–5日，平均4.4日，觀察33粒卵能順利發育為成蟲之羽化率為66.7%。雄蟲壽命平均30日以上，雌蟲壽命可達50日以上，平均25.3日，一生總產卵數多者550粒以上，平均258.7粒 (表2)。

葉蚤在一般養蟲環境下即可順利繁殖，卵經過3週即可羽化成蟲，成蟲在一個月內持續產卵，其發育速度快，產卵能力強，且集體飼養時無自殘行為。而長梗滿天星易於扦插繁殖，葉蚤食物不虞匱乏，所需飼養成本不高，故評估本種葉蚤經室內飼養繁殖後，於田間釋放作為生物防治之可行性高。

田間調查長梗滿天星主要發生於台灣中部與北部，環境適應性極強，能同時生存於陸地

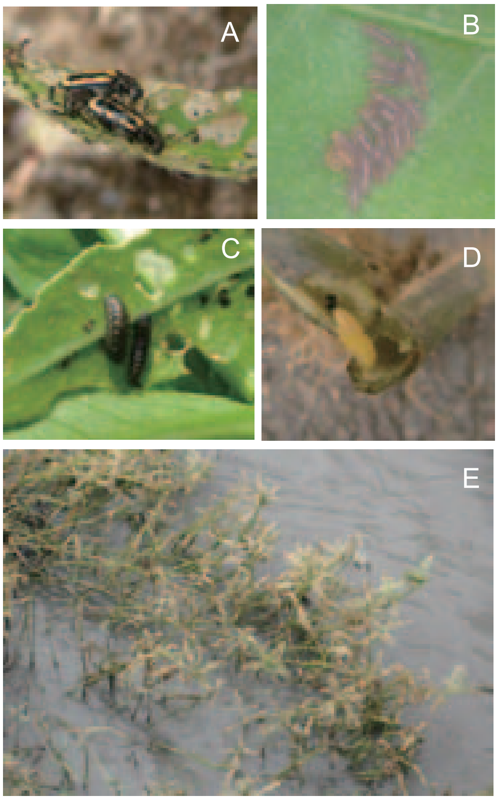

圖 1. 雙條長葉蚤各時期。(A) 成蟲；(B) 卵堆；(C) 幼蟲；(D) 蛹；及 (E) 被被葉蚤吃到枯黃的長梗滿天星。

Fig.1. Different stages (A–D) of alligatorweed flea beetle, *Agasicles hygrophila*. (A) Adults; (B) Egg mass; (C) Larvae; (D) Pupa, and (E) withered alligator weeds caused by heavy infestation of flea beetles.

表 1. 雙條長葉蚤未成熟期發育日數

Table 1. The immature development of eggs, larvae and pupae of *Agasicles hygrophila* [z]

		Larva					
Duration (d)	Egg	1st instar	2nd instar	3rd instar	Total	Pupa	Emergence (%)
Min.	4	2	2	3	11	4	—
Mean ± SD	4.9 ± 0.2	3.6 ± 0.9	3.5 ± 1.7	5.9 ± 1.5	12.3 ± 1.6	4.4 ± 0.5	66.7
Max.	5	5	7	10	18	5	—

[z] 33 eggs were observed under conditions at 26–27℃ and 24 L photoperiod.

表 2. 雙條長葉蚤成蟲壽命及繁殖力

Table 2. Longevity and fecundity of adults of *Agasicles hygrophila* [z]

	Longevity		
Duration (days)	Male	Female	No. eggs laid/female
Min.	11	3	0
Mean ± SD	29.0 ± 12.4	25.3 ± 15.3	258.7 ± 204.2
Max.	55	57	569

[z] 8 males and 12 females were observed under conditions at 26–27℃ and 24 L photoperiod.

與水域，陸生者存在於水分充足的公園草坪、菜園、路邊等；水生者發現於池塘、水澤、灌溉溝渠及水田。葉蚤分布自中部至北部均有，但並非全面性普遍發生，僅發現於部分的水澤地與溝渠。自南投到北海岸所調查的37處長梗滿天星中，發現8處有雙條長葉蚤，占長梗滿天星發生比率之21.6%，分布於南投草屯、台中霧峰、苗栗公館與三灣、新竹竹東與關西，台北淡水等地 (圖2)。當生於濕地或水域中植株被害嚴重，陸生者雖為水生植株延續而生，距離僅數公尺，卻可免於葉蚤取食或棲息，顯示本種葉蚤適於水生環境，僅對於水生長梗滿天星有取食能力。國外資料亦顯示以往利用葉蚤生物防治成功的例子均為水生滿天星，陸生者則無防治效果 (Forno & Julien 2000)。

在不同季節中，自然發生之葉蚤族群會有密度上的差別。本種葉蚤發育適溫為20–30℃之間，高溫或低溫時其發育與繁殖力差 (Steward *et al.* 1999)。以台灣的氣候與溫度，本蟲在春、秋季節因溫度適宜，生存與繁殖力強，在田間會以較高密度存在，例如4–6月田間常可看到

圖 2. 雙條長葉蚤在台灣中、北部長梗滿天星上之發生。

Fig. 2. Occurrence of alligatorweed flea beetle, *Agasicles hygrophila* in central and northern Taiwan.

寄主植物被高度取食之群落。幼蟲啃食嫩葉表面，通常只吃食一層葉表，留下多數圓點形白色薄膜狀食痕；成蟲取食量稍大，會直接造成葉片較大缺口與孔洞。葉蚤偏好集中於幼嫩的新葉，密度高時會對植株造成嚴重傷害，芽頂萎縮甚至全株枯黃 (圖1E)。

當進入夏季，溫度逐漸升高，葉蚤密度與活動力隨之降低，調查中仍可見有部分葉片嚴重被食害，但長梗滿天星的生長隨溫度升高而旺盛，此時葉蚤取食量不足以使植株枯死。只是葉片的被害多少會降低生長勢，因而削弱其與周邊其他植物之競爭力，對於其在環境中的發生與擴張會不同程度之影響。盛夏溫度常達35℃左右，此種高溫不適合本葉蚤生長，葉蚤繁殖與生存能力均低，密度銳減，長梗滿天星上已難得見到葉蚤蹤跡，此時卻是多年生之長梗滿天星生長最盛時期，之前曾受害之植株可再恢復旺盛生機。

台灣未曾引進雙條長葉蚤，其存在可能是由於隨進口植物進入，或是自然擴散。因其寄主植物僅有長梗滿天星一種，在相似水域環境中的其他經濟植物種類少，故隨進口農產品夾帶進入的可能性低；但鄰近之中國大陸的福建、廣東均有本種葉蚤存在 (Ma *et al.* 2003)，泰國亦曾經人為釋放過 (Napompeth 1981; Waterhouse 1994)，故不排除自大陸東南沿海或東南亞隨氣流擴散而來的可能。

雙條長葉蚤之利用開始於美國自阿根廷之引進，台灣由鄰近地區自然侵入之葉蚤極可能是間接的源於溫帶，該品系適應之溫度範圍有限，以致於某些季節，族群密度降低；台灣夏季有長時間日夜溫度均高，自然發生的葉蚤對於長梗滿天星難以產生實際防治效果。在有特殊需要之情況下，可考慮配合人為室內飼養，集中大量釋放蟲體，以擴增田間密度的方式達到防治效果。葉蚤之溫度適應是影響生物防治成功之重要因子，台灣若經過測試與選汰後，引入耐高溫環境之蟲系，亦有可能改變目前葉蚤與長梗滿天星間之關係，達到較理想之防治效果。

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Occurrence of *Agasicles hygrophila* (Coleoptera: Chrysomelidae) and Its Potential for Biocontrol on Alligatorweed, *Alternanthera philoxeroides*[1]

Chin-Ling Wang[2,4], Yu-Chen Hsieh[3], Feng-Chyi Lin[2], and Mou-Yen Chiang[3]

Abstract

Wang, C. L., Y. C. Hsieh, F. C. Lin, and M. Y. Chiang. 2012. Occurrence of *Agasicles hygrophila* (Coleoptera: Chrysomelidae) and its potential for biocontrol on alligator weed, *Alternanthera philoxeroides*. J. Taiwan Agric. Res. 61:75–79.

The alligatorweed flea beetle, *Agasicles hygrophila* Selman & Vogt (Coleoptera: Chrysomelidae) is monophagous on alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb.. Both larvae and adults feed on leaves and may cause serious damage on plants when the insect population is high. The flea beetle was reared under standard insectary conditions at 26–27°C, and a 24 hour photoperiod (continuous light). The egg incubation period was about 5 days and the larval developmental time was 12–13 days. The pupal stage was 4–5 days. Average longevity of female adults was 25.3 days, with an average of 258 eggs laid per female. Results of field surveys showed that the flea beetle occurred locally on alligator weed, with higher population densities in spring and autumn. Heavily infested plants became withered and died. High temperatures in the summer was favorable for the growth of alligator weeds but was unfavorable for the development and reproduction of the flea beetle. Although these alligatorweed flea beetles may reduce growth vigor of alligator weed, the natural population of this insect had little functional value for control of this weed due to differences in optimal growth temperature. Mass rearing and field release of the flea beetles may increase population densities for the control purposes. Importation of a heat tolerant strain may also alter the current relationship between the flea beetle and alligator weed.

Key words: *Agasicles hygrophila*, *Alternanthera philoxeroides*, Taiwan, Biocontrol.

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「台灣農業研究」稿約

一、 本刊爲行政院農業委員會農業試驗所發行之學術性季刊，刊登農業試驗研究相關之研究稿件：

論　　說：由本所邀請專家撰寫。

研究報告：關於農業科學未曾發表之研究報告，按題目、作者、摘要、前言、材料與方法、結果、討論、結論 (一般略去)、誌謝 (可略去)、引用文獻等依序撰寫，圖與表附於其後，全文以不超過 10印刷頁爲原則。

研究簡報：關於農業科學未曾發表之簡短原始研究報告，不須分章節撰寫，但須附中英文摘要，全文以不超過 4印刷頁爲原則。

二、 稿件應以中文或英文撰寫。來稿請送原稿及電子檔案各一份 (內各附照片原圖，繪製圖版可附原圖與影印圖片二份)。文稿以 A4大小之白色紙張 (長 29.6公分，寬 21公分) 兩倍行高 (double space) 打字， 12號字體，左邊對齊，版面應上下預留 3公分，左右 2公分之空間，並自第二頁起在右下角以阿拉伯數字註明頁數。撰寫時，中文以細明體、英文以 Times New Roman字體打字；英文字距一格，句距二格。審查接受之稿件，隨文請附紙本文稿一份與電子檔案，以利送審及電腦排版等作業。

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題　　目：宜簡短，能充分反應文旨，並附簡題 (Running title) (中文 12字；英文 6字以內爲原則)。英文題目除介系詞、連接詞等字外，每字第一字母應大寫。題目附小註，說明研究報告編號。

作　　者：作者姓名置中，中文名字間空一格；英文姓名則名在前姓氏在後，作者二人或二人以上時，以 “，” 相隔，最後一人前加 “and” 字樣，其表示方法如 Chen-Seng Huang, Chen-Chang Chen, and Chyr-Guan Chen。每一作者須附小註，以說明所屬機關單位，通訊作者須附電子郵件信箱與傳真機號碼。題目與作者同置第一頁。

摘　　要：不分段落、宜簡潔明確。摘要之前應加作者、年代、題目、期刊、及卷期，摘要之後應附關鍵詞 (Key words)，關鍵詞不超過 5個，每個英文關鍵詞第一字母應大寫。摘要以不超過 500字爲原則。以中 (英) 文發表者須附英 (中) 文摘要，置於本文之後，且內容須與中 (英) 文摘要相符，並附稿件編號、作者、通訊作者之小註。

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